35. The method of claim 33 wherein said autoimmune disease is selected from the group comprising: inflammatory bowel disease, rheumatoid arthritis, type 1 diabetes mellitus, lupus erythematosus, multiple sclerosis, sarcoidosis, autoimmune thyroiditis, allergic rhinitis, and asthma.

REMARKS

Claims 24-32 are pending.

New claims 33-35 have been added.

Support for the addition of claim 33, and dependent claims 34-35 can be found in the specification on page 5 lines 17-20, which state "As used herein, the term autoimmune disease refers to such diseases as IBD rheumatoid arthritis, type 1 diabetes mellitus, lupus erythematosus, multiple sclerosis, sarcoidosis, autoimmune thyroiditis, allergic rhinitis, and asthma." Support is also found at lines 24-26 which state "As used herein, the term excessive or aberrant immune response refers to a Th1 response in which the activity of T helper 1 cells is elevated in an individual relative to the activity of such cells in an individual who is not affected by the disease.", and page 6, line 10 "[W]herein the IBD is Crohn's disease or ulcerative colitis".

Rejections under 35 U.S.C. 102(b)

Claims 24-32 are rejected under 35 U.S.C. 102(b) as being anticipated by Kullberg et al. The Applicants traverse this rejection

The Examiner states that Kullberg et al. teaches a method of screening a helminthic parasite preparation for components which induce down regulation of Th1 responses (Abstract).

The Examiner also states that Kullberg et al. teaches assaying the preparation in vitro and in vivo (Materials and Methods).

The Examiner then states that "The method of screening helminthic parasite preparation of Kullberg et al. does not have exactly the same steps as [the] claimed method. However the method of Kullberg et al. screens [the] same helminthic parasite preparation components and the screened components have the same biological property as [the] claimed method [and] can screen- helminthic parasite preparation components which are capable of reducing an excessive Th1 response. Thus, Kullberg et al. meet the limitation of the claims."

The Applicants submit that Kullberg et al., does not disclose a method of screening helminthic parasitic compounds for components that reduce an excessive Th1 immune response, as claimed in the instant application. The Kullberg study investigates the effect on antigenic challenge by sperm whale myoglobin to mice treated with the Schistosoma mansoni parasite. The first sentence in the Results section states that "It was important in comparing cytokine responses of SC from infected and uninfected animals, to know how schistosomiasis would affect the composition of cells in the spleen". The first sentence in the Discussion reads "In this report, the effect of S. mansoni infection on cytokine and antibody responses to a non-parasite

Ag. SwMb, was analyzed". The Kullberg paper is interested in determining the effect of Schistosoma infection on particular immune responses and cell types in mice, not, as the Applicants claim—a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 response.

The Applicants further submit that the Examiners application of 35 U.S.C. 102(b) is a misapplication of law. To find anticipation of claims, the prior art embodiments must possess the elements expressly recited in the claims, *E.I. du Pont de Nemours & Co. v. Philips Petroleum Co.*, 7 U.S.P.Q.2d 1129 (Fed. Cir. 1988). For a prior art reference to anticipate, every element of the claimed invention must be identically shown in a single reference, *In re Bond*, 15 U.S.P.Q2d, 1156 (Fed Cir. 1990). "[A]ny degree of physical difference, however slight, invalidates claims of anticipation". *Ultradent Products, Inc. v. Life-Like Cosmetics, Inc.*, 39 U.S.P.Q2d 1969 (Utah 1996). The Applicants assert that the present claims are directed toward a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response said method comprising the step of assaying a fraction of a helminthic parasite preparation to detect biological activity that reduces an excessive Th1 immune response.

The Kullberg et al., reference does not disclose a method of screening helminthic parasite preparation for "one or more components" that "reduces an excessive Th1 immune response" as claimed in claims 24-32. Kullberg et al., does not disclose a method of screening the same by "assaying a fraction" of a helminthic parasite preparation as claimed in claims 24-32. Kullberg et al., does not disclose "assaying a fraction" of a helminthic parasite preparation as claimed in claims 24-28. Lastly, with respect to claims 29 and 30, Kullberg et al., does not disclose "chromatographic separation techniques" to screen for components of a helminthic parasitic preparation that reduce an excessive Th1 immune response.

The instant claims thus are not anticipated by Kullberg et al., as the reference does not disclose each element of the claimed invention. As such, the Applicants respectfully request withdrawal of the rejection under 35 U.S.C. 102(b).

Rejections under 35 U.S.C. 102(e)

The Examiner states that claims 24-31 are rejected under 35 U.S.C. 102(e) as being anticipated by Moyle et al. The Applicants traverse this rejection.

The Examiner notes that "Claims 24-31 are drawn to a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response which is indicated by modulation of inflammation".

The Applicants respectfully point out that the Examiner has misstated the claimed invention. The claims are directed toward a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response, **said method comprising the step of assaying a fraction of a helminthic parasite preparation to detect biological activity that reduces an excessive Th1 immune response.**

The Examiner states that "Moyle et al., teach[es] a method of screening a helminthic parasite preparation for components which act as anti-inflammatory compounds [and] function

as inhibitor[s] of neutrophil activation (Abstract and columns 2, lines 61-67, column 3, lines 1-25, column 13, lines 15-33, and column 7, lines 50-65) – a[s] indicative of [a] reduced excessive Th1 immune response". The Examiner further states that "Moyle et al., also teach using chromatographic separation techniques for the screening (column 7, lines 50-65 and Examples) and assaying the preparation in vitro (Examples A and B)."

Moyle et al., shows that a 38-kDa protein from hookworm inhibits neutrophil adhesion to endothelial cell monolayers or to plastic dishes, and neutrophil aggregation in vitro. They called this protein neutrophil inhibitory factor. Moyle et al., does not anticipate the claimed invention because neutrophil activity is not an indicator of an excessive Th1 immune response, and thus inhibition of neutrophil binding to endothelial monolayers or to plastic in vitro, or inhibition of neutrophil aggregation in vitro is not characteristic of inhibition of an excessive Th1 immune response. The instant claims refer to a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response, the method comprising the step of assaying a fraction of a helminthic parasite preparation to detect biological activity that reduces an excessive Th1 immune response.

First, Moyle et al., does not teach assaying a parasitic preparation fraction for a component that reduces an excessive Th1 immune response. An "excessive Th1 immune response" is defined in the above referenced patent application as "[A] Th1 response in which the activity of T helper 1 cells is elevated in an individual relative to the activity of such cells in an individual who is not affected by the disease" (page 5, lines 24-26). Moyle et al. teach assays in which a 38-44 kDa glycoprotein of Ancylostoma caninum inhibits isolated neutrophils from adhesion to endothelial cell monolayers or to plastic dishes, and aggregation with other neutrophils. The definition of "excessive Th1 immune response" in the above referenced patent application does not include neutrophil activity or neutrophil adhesion to endothelial cells, or neutrophil adhesion to plastic, or neutrophil aggregation as indicative of an excessive Th1 response. Moreover, neutrophil activation is not characteristic of the development of immunological diseases like Crohn's disease, rheumatoid arthritis, or multiple sclerosis, etc. The examiner is referred to the enclosed 132 declaration by the inventors of the above-referenced patent application, wherein it is stated and Exhibit C-E demonstrate that neutrophil activity is not relevant to or an indicator of a Th1 immune response, and thus assaying the 38-44 kDa protein disclosed in Moyle et al. inhibit or of neutrophil activation is not the same biological assay as claimed in claim 33. That is, claim 33 cannot encompass the assay presented in Moyle et al. because neutrophil activity (or inhibition thereof) is not relevant to an excessive Th1 immune response (or inhibition thereof). The Applicants respectively request that the 102 rejection be withdrawn.

Rejections under 35 U.S.C. 103

The Examiner states that claims 24-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Moyle et al. and Kullberg et al.

The Examiner states that Moyle et al. teaches a method for screening a helminthic parasite preparation as applied in the 102 rejection. The Examiner further states that Moyle et al. "clearly teach that the screened components can be used in vivo (column 15, lines 31-67) and Kullberg et al. teach a method of screening a helminthic parasite preparation for components

assaying the preparation in vivo." The Examiner states that it would have been obvious "to assay the preparation of Moyle et al. in vivo as taught by Kullberg because the known benefit of the components preparation of Moyle et al. will be useful in treating a variety of clinical disorders (column 13, 15-34)". The Applicants traverse this rejection.

The Kullberg et al., paper teaches that Schistosoma infection changes the nature of the humoral immune response to challenge by a foreign antigen. It further teaches that this altered immune response originates from splenic APC and CD4+ T cells. The Moyle et al., patent teaches that neutrophils respond to soluble inflammatory mediators released by cells at the site of injury (column 1, lines 13-15). Moyle et al., further teaches that this 38-44 kDa glycoprotein acts upon isolated neutrophils in that it "inhibits neutrophil activity, particularly neutrophil adhesion to vascular endothelial cells" (column 5, line 12-15), specifically HUVEC monolayers (column 23, lines 10-35) and neutrophil binding to plastic (column 23, lines 36-64) and neutrophil aggregation (column 23, lines 65-67 through column 24 lines, 1-10). Applicants submit that the combination of Moyle et al., in view of Kullberg et al., does not provide the invention as claimed i.e., a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response, said method comprising the step of assaying a fraction of a helminthic parasite preparation to detect biological activity that reduces an excessive Th1 immune response.

The Moyle et al., and Kullberg et al., references, when combined, do not provide the claimed invention. The Moyle et al. patent does not teach screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response. The Kullberg et al., paper does not teach screening a helminthic parasite preparation for one or more components that reduces an excessive Th1 immune response. The Moyle et al., patent does not teach assaying a fraction of a helminthic parasite preparation to detect biological activity that reduces an excessive Th1 immune response. The Kullberg et al., paper does not teach assaying a fraction of a helminthic parasite preparation to detect biological activity that reduces an excessive Th1 immune response.

The Applicants submit that their invention is directed toward a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response, the method comprising the step of assaying a fraction of a helminthic parasite preparation to detect biological activity that reduces an excessive Th1 immune response. The Applicants submit that the Examiner has not identified how the cited references, when combined, disclose all of the elements of the claimed invention. "To evaluate obviousness, a comparison must be made between the prior art as a whole and the claimed subject matter as a whole.", *In re Langer and Haynes,* 465 U.S.P.Q. 169, 171 (C.C.P.A. 1972).

Moyle et al. discloses that GM-CSF, IL-8, and TNF-α are all factors that would activate neutrophils (column 2, lines 20, and 37-42). Moyle et al. teaches the isolation of a protein which "specifically inhibits neutrophil activity" because it "inhibits neutrophil adhesion to vascular endothelial cells and homotypic neutrophil aggregation" (column 8, lines 34-38). The Moyle et al. patent is silent as to a Th1 immune response, and an excessive Th1 immune response. Thus one skilled in the art would not expect that fractionating a parasite extract and assaying for neutrophil inhibition via the in vitro isolated neutrophil assays taught in Moyle et al. would have any effect with respect to reducing an excessive Th1 immune response.

The Kullberg et al. reference actually teaches away from the Applicants' invention. The Applicants' disclose the detection of cytokine levels and immunoglobulin isotype switching as possible indicators of a component that reduces an excessive Th1 immune response . Kullberg et al., states on page 3268, second column lines 33-39 that "Because of the known influences of cytokines on isotype selection, serum antibodies specific for SwMb in infected and uninfected animals were analyzed for isotype differences. SwMb-specific IgG1 and IgG2a antibodies showed similar decreases, suggesting no selective changes in isotypes between infected and uninfected mice". Based on the Kullberg et al., reference, it would not be obvious to one skilled in the art to screen helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response, by evaluating the cytokine levels and immunoglobulin isotypes.

The Applicants assert that the Examiner has not proven a *primae facie* case of obviousness in the instant application. "While the test for establishing an implicit teaching, motivation, or suggestion is what the combination of these...[w]ould have suggested to those of ordinary skill in the art, the two statements can not be viewed in the abstract. Rather, they must be considered in the context of the teaching of the entire reference. [P]articular findings must be made as to the reason the skilled artisan, with no particular knowledge of the claimed invention, would have selected these components for combination in the manner claimed" *In re Werner Kotzab*, 55 U.S.P.Q.2d 1313 (C.A.F.C. 2000). "Before obviousness may be established, the Examiner must show that there is either a suggestion in the art to produce the claimed invention or a compelling motivation based on sound scientific principles.", *Ex parte Kranz,* 19 U.S.P.Q2d 1216, 1218 (B.P.A.I. 1990). "In proceedings before the PTO, the Examiner bears the burden of establishing a prima facie case of obviousness based on the prior art. The Examiner can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references.", *In re Fritch*, 23 U.S.P.Q2d 1780, 1783 (Fed. Cir. 1992). In the instant case neither reference alone or in combination suggests the Applicants' method of screening parasitic preparations for components that reduce excessive Th1 immune responses.

The Declaration of Joel Weinstock and David Elliott, executed and submitted under 37 C.F.R. 1.132, is offered as evidence of the non-obviousness of the claimed invention. The Declarants assert in paragraph 4 of the Affidavit that neutrophil activation is not characteristic of the development of immunological diseases like Crohn's disease, rheumatoid arthritis, or multiple sclerosis, for example. Moreover, neutrophils do not have a critical role in the maintenance of ongoing chronic disease activity, of aberrant immunological diseases. The method of Moyle, et al., would not allow identification in a parasite extract of a fraction that inhibits an excessive of Th1 immune response. The Applicants also attest in paragraph 5 that there is a long-felt need for components that can treat an excessive/aberrant Th1 immune response as many diseases involving an aberrant/excessive Th1 response have not been successfully treated in the human population. The Applicants further attest that there continues to be a need for components that can effectuate such treatment, and thus a need for methods of assaying a fraction of a helminthic parasite preparation for reducing an excessive Th1 immune response, as claimed in the instant application. The Declarants further attest in paragraph 6 that the proposed use of components that can treat an excessive/aberrant Th1 immune response has generated noteworthy public interest and acclaim. The Declarants further state that the claimed method of screening a helminthic parasite preparation for one or more components that reduce an

excessive Th1 immune response, comprising the step of assaying a fraction of a helminthic parasite preparation to detect biological activity that reduces an excessive Th1 immune response, does satisfy the long-felt need for a method of screening these components.

The Applicants respectfully state that 35 U.S.C. 103 is not a bar to patentability of the claimed invention.

In view of the above, it is submitted that all of the claims in the instant application are in a condition for allowance. Such action is respectfully requested.

Respectfully submitted,

Kathleen M. Williams
Reg. No. 34,380
Attorney of Record
Palmer & Dodge, LLP
One Beacon Street
Boston, Massachusetts 02108-3190
(617) 573-0100 (telephone)
(617) 227-4420 (facsimile)

Dated: 9/12/06

Attorney Docket 27045/1020 (formerly: 3948/79934)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#10 9/23/00 T.Gray

Application of:	Weinstock and Elliott		
Serial No.:	09/362,598	Examiner:	Lee, L.
Filed:	07/28/99	Art Unit:	1645
Entitled:	USE OF PARASITIC BIOLOGICAL AGENTS FOR PREVENTION AND CONTROL OF AUTOIMMUNE DISEASES		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF JOEL V. WEINSTOCK AND DAVID E. ELLIOTT UNDER 37 C.F.R. § 1.132

I, Joel V. Weinstock, hereby declare that:

1. I am on of the inventors in the above named patent application. I am employed by the University of Iowa as a Physician-Scientist in the Department of Internal Medicine. I received a M.D. from Wayne State University in 1969. I have been employed by the University of Iowa since 1986.

I, David E. Elliott, hereby declare that:

2. I am one of the inventors on the above named patent application. I am employed by the University of Iowa as a Physician-Scientist in the Department of Internal Medicine. I received a Ph.D. in Immunology/Microbiology from Wayne State University in 1985. I received a M.D. from Wayne State University in 1988. I have been employed by the University of Iowa since 1991.

3. We have read the Office Action mailed April 12, 2000, in the above referenced patent application, and the references cited by the Examiner.

4. Moyle et al., teaches that a 38-44 kDa polypeptide from a hookworm inhibits neutrophil adhesion to a vascular endothelial monolayer in vitro, to plastic, and inhibits neutrophil aggregation in vitro. However, neither these activities of neutrophils, or neutrophil

activation in general, are characteristic of an excessive Th1 immune response. Nor do neutrophils play a critical role in immunological diseases like Crohn's disease, rheumatoid arthritis, or multiple sclerosis. Moreover, neutrophils do not have a critical role in the maintenance of ongoing chronic disease activity of aberrant immunological diseases. The method of Moyle, et al., therefore, using in vitro inhibition of neutrophil adhesion, would not allow identification in a parasite extract of a fraction that inhibits an excessive Th1 immune response.

The scientific literature clearly demonstrates that neutrophil activation is not an indicator of an excessive Th1 immune response, nor is inhibition of neutrophil activation equivalent to studying inhibition of an excessive Th1 immune response.

Attached as Exhibit C is a review article by Falcone et al. This article described the cells which are relevant to a Th1 immune response, that is, T cells, macrophages, and other antigen presenting cells. Neutrophils, are not considered to be relevant to such a response, and consequently, the article ignores neutrophils.

Attached as Exhibit D is an article by Brown et al. describing a rat experimental colitis model and the immune response involved in this disease model. The article states on page G588: "neutrophil depletion has not been shown to effectively reduce acetic acid or TNBS-induced colonic damage" and "induction of neutropenia ... did not ameliorate the TNBS-modified colonic injury." Brown et al. states "in PMA-induced colitis, neutropenia has been shown to be ineffective in reducing, the degree of damage," and on page G589: "this study also supports the proposal that neutrophils do not mediate the inflammatory effects of TNBS and are not involved in the colonic response to PKC activation." Thus, Exhibit D demonstrates that neutrophils are not involved in and thus are not relevant to a Th1 mediated immune response. Inhibition of neutrophil activity thus is not relevant to inhibition of a Th1 immune response.

Attached as Exhibit E is an abstract by Melarange et al. concluding that "neutrophils do not contribute to gastrointestinal ulceration and blood loss induced by nonsteroidal anti-inflammatory drugs. Furthermore, in contrast with previous studies our results provide no evidence that neutrophils contribute to indomethacin-induced acute gastric erosion formation." Exhibits C-E thus demonstrate that neutrophils are not involved in a Th1 immune response.

5. There exists a long-felt need for a method of treating an excessive immune response including an aberrant/enhanced Th1 response. We as physicians recognize, and it is well recognized in the medical community, that many diseases involving an aberrant/excessive Th1 response have not been successfully treated in the human population. There continues to be a need for such a treatment. More specifically, there continues to be a need for components that can effectuate such treatment, and thus a need for methods of assaying a fraction of a helminthic parasite preparation for reducing an excessive Th1 immune response, as claimed in the instant application.

6. Post-filing date public disclosure of our related invention designed to treat an aberrant/enhanced Th1 immune response by administration of a helminthic parasite preparation generated both widespread public interest in the invention and acclaim for that invention, including inquiries from people wishing to obtain treatment or participate in further studies. This

is demonstrative of the noteworthy public interest in and acclaim for the success of use of that claimed invention in treatment. This is also demonstrative of the long-felt need for additional components that can effectuate such treatment.

7. The discovery that a helminthic parasite preparation is useful to treat an excessive Th1 immune response, e.g., as found in chronic inflammatory diseases, satisfies the long-felt need, as is evidenced by Exhibits A and B, which is a sample of the numerous publications, news coverage, and public comments, all evidencing significant public interest in, and acclaim for this treatment. The claimed method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response, comprising the step of assaying a fraction of a helminthic parasite preparation to detect biological activity that reduces an excessive Th1 immune response, also satisfies the long-felt need.

8. A helminthic parasite preparation is useful to treat an excessive Th1 immune response, and the patients treated with this therapy do not suffer the dangerous side effects that are seen with other therapies. Current drugs, like Prednisone, Azathioprine, and Cyclosporine, for example, all have serious side effects. More specifically, there is a long-felt need for a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response, as claimed in the above application.

We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

9/12/00
Date

Joel V. Weinstock
Joel V. Weinstock, M.D.

9/12/00
Date

David E. Elliott
David E. Elliott, M.D., Ph.D

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/362,598	07/28/99	WEINSTOCK J	27045/1020

HM12/0412

KATHLEEN M WILLIAMS
BANNER & WITCOFF LTD
28 STATE STREET
28 FLOOR
BOSTON MA 02109

EXAMINER	
LEE,L	
ART UNIT	**PAPER NUMBER**
1645	8

DATE MAILED: 04/12/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trad marks

Office Action Summary

Application No.	Applicant(s)
09/362,598	Weinstock et al
Examiner	**Group Art Unit**
Li Lee	1645

☒ Responsive to communication(s) filed on *Jan 14, 2000*

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claim

☒ Claim(s) *1-32* is/are pending in the applicat

Of the above, claim(s) *1-23* is/are withdrawn from consideration

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *24-32* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☒ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *7*

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Election/Restriction

1. Applicant's election without traverse of Group VII, claims 24-32 in Paper No. 7 is acknowledged.

Information Disclosure Statement

2. Items listed on form PTO-1449 filed on Sep 13, 1999 have be considered by the examiner.

Drawings

3. This application has been filed with informal drawings which are acceptable for examination purposes only. The drawings are objected to by the draftsperson under 37 C.R.F. 1.84 or 1.152. See PTO-948 for details. Correction of the noted defects can be deferred until the application is allowed by the examiner.

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371© of this title before the invention thereof by the applicant for patent.

5. Claims 24-32 are rejected under 35 U.S.C. 102(b) as being anticipated by Kullberg et al (J Immunol May 15, 1992).

Claims 24-32 are drawn to a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response.

Kullberg et al teach a method of screening a helminthic parasite preparation for components which induces a down-regulation of Th1 responses (Abstract). Kullberg et al also teach assaying the preparation in vitro and in vivo (Materials and Methods). The method of screening helminthic parasite preparation of Kullberg et al does not have exactly same steps as claimed method. However, the method of Kullberg et al screens same helminthic parasite preparation components and the screened components have same biological property as claimed method can screen- helminthic parasite preparation components which are capable of reducing an excessive Th1 immune response. Thus, Kullberg et al meet the limitation of the claims.

6. Claims 24-31 are rejected under 35 U.S.C. 102(e) as being anticipated by Moyle et al. (US 5,708141, Jan 13, 1998).

Claims 24-31 are drawn to a method of screening a helminthic parasite preparation for one or more components that reduce an excessive Th1 immune response which is indicated by modulation of inflammation (at the specification, page 21, second paragraph).

Moyle et al teach a method of screening a helminthic parasite preparation for components which act as anti-inflammatory compounds function as inhibitor of neutrophil activation (Abstract and columns 2, lines 61-67, column 3, lines 1-25, column 13, lines 15-33, and column 7, lines 50-65) - a indicative of reduced excessive Th1 immune response. Moyle et al also teach using chromatographic separation techniques for the screening (column 7, lines 50-65 and Examples) and assaying the preparation in vitro (Examples A and B).

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. Claims 24-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Moyle et al. (US 5,708141, Jan 13, 1998) and Kullberg et al (J Immunol May 15, 1992).

Moyle et al teach a method of screening a helminthic parasite preparation for components as applied in 102 rejection above. Moyle et al do not expressly assaying the preparation in vivo. However, Moyle et al clearly teach that the screened components can be used in vivo (column 15, lines 31-67) and Kullberg et al teach a method of screening a helminthic parasite preparation for components assaying the preparation in vivo.

Therefore, it would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to assay the preparation of Moyle et al in vivo as taught by Kullberg et al. because the known benefit of the components preparation of Moyle et al will be useful in vivo in treating a variety of clinical disorders (column 13, 15-34).

Thus, the claimed invention as a whole was clearly prima facie obvious.

Status of Claims

9. No claims are allowed. All claims stand rejected.

Any inquiry of a general nature or relating to the status of this general application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Papers relating to this application may be submitted to Technology Center 1600, Group 1645 by facsimile transmission. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). Should applicant wish to FAX a response, the current FAX number for Group 1600 is (703) 308-4242.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Li Lee whose telephone number is (703) 308-8891. The examiner can normally be reached on Monday-Friday from 8:30 AM to 5:00 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached at (703) 308-3995.

Li Lee
April 5, 2000

ALBERT NAVARRO
PRIMARY PATENT EXAMINER

EXHIBIT "A"

Human studies in IBD

The initial studies were done on patients with refractory Crohn's disease and ulcerative colitis. They had failed treatment with powerful anti-inflammatory medications like prednisone and azathioprine. The patients were give only one low dose of the organism *T. suis*. Our first objective was just to show that low-dose colonization with this helminth would cause no harm.

Table 4 below shows the effect of helminthic therapy on our first 5 patients with Crohn's disease treated with *T. suis*. All got substantially better. It took about 2-3 weeks to respond. The Crohn's disease activity index is a well-accepted measurement of disease activity in Crohn's disease. A drop below **150** on this score is considered a complete remission. An improvement of 70 points or better is considered highly significant. The IBDQ is an inflammatory bowel disease quality of life indicator. The higher the number the better, with a score of **170** or higher considered remission. All patients perceived substantial improvement in their sense of well being. The ESR (erythrocyte sedimentation rate) and C-reactive protein are serological tests. These perimeters climb with systemic inflammation. Most patients showed improvement in one or more of these tests indicative of improvement in their disease.

Table 4

	CDAI		IBDQ		ESR		C-reactive Protein	
CD patients	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	250	147	115	198	19	6	0.9	<0.5
2	341	19	127	221	6	1	<0.5	<0.5
3	176	135	118	175	41	34	9.1	2.4
4	565	413	54	104	30	20	0.7	<0.5
5	273	130	105	200				

Table 5 shows results in two patients with ulcerative colitis. Both patients substantially improved within 4 wks.

Table 5

	IBDQ		Stools/week		ESR		C-reactive Protein	
UC patients	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	138	191	42	26	14	9	0.9	<0.5
2	166	188	16	9	19	19	0.9	0.6

The patients remained in remission for 1-5 months. Two patients with CD and two with UC were retreated after relapse and again went into remission **(Figure 11).** No patients developed adverse reactions to this therapy. We are now organizing a 'double-blind', controlled study.

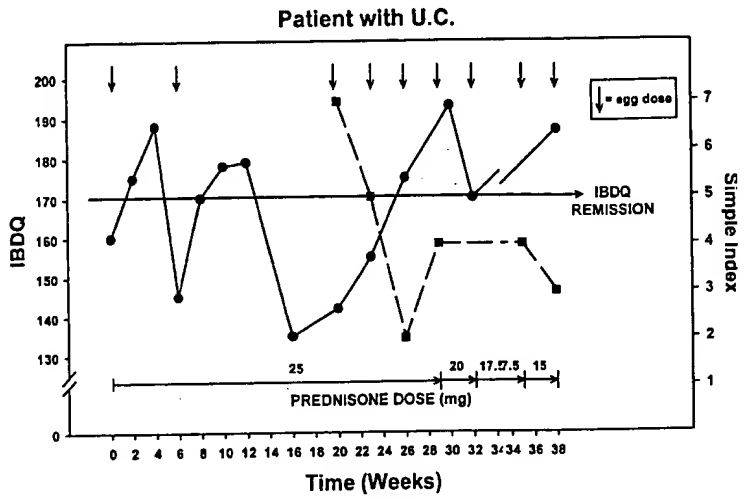


Figure 11. This is the clinical coarse of a patient with refractory ulcerative colitis who received repeated doses of *T. suis* ova for over >6 months. A remission is defined as an IBDQ score >170. The horizontal line next to the IBDQ indicates the point of remission. The arrows indicate when he received ova. The dashed line is his 'Simple Index' score. In the Simple Index, the lower the number the better. The duration of his response was about 6 wk for each of the first two treatments. Subsequently, he was place on every 3-wk dosing. He has remained in remission and weaned from his steroids. The duration of his remission is now >9 months.

COPY

August 13, 1999

TO: Diana Lundell
Stacy McGauvran-Hruby
Shannan Janney
Tom Moore

FR: Lorna Bennett

RE: Weinstock Media Coverage 8/4 – 8/13

cc: Julie Gilman
Kristine Wilber

Attached please find recent media coverage of Weinstock's worm study. Print coverage we obtained via a Lexus/Nexus search. Comprehensive broadcast coverage reports were provided by Video Monitoring Services (VMS).

Print Coverage

August 5

- *Reuters (London)* – "Hard to swallow? Worms could cure bowel disorders"
- *UPI* – In *Domestic News/Health Tips*
- *Calgary Herald* (Early and Final) – "Doctors put parasites put to work"
- *The Herald (Glasgow)* – "Worms bring cure for bowel disease"
- *The Journal (Newcastle)* – "Worms link to bowel disease"
- *The Ottowa Citizen* – In *News of the World*
- *The Times (London)* – "Patients helped by dose of worms"
- *The Vancouver Sun* – "Belly full of worms aids bowel illnesses"
- *Western Daily Press* – "Time for worms to return?"

August 6

- *The Independent (London)* – "Science update: Inflammatory bowel disease"

August 7

- *AP State and Local Wire* – "Healthful benefits found in worms"
- *New Scientist* – "Wonderful worms"

August 8

- *Telegraph Herald (Dubuque)* – In *Iowa Briefs*

August 9

- *The Irish Times* – In *Lifelines*

Broadcast Coverage

August 4
- KCOP-TV (UPN) – Los Angeles
- WLTV-TV (Univision) – Miami

August 6
- KMBC-TV (ABC) – Kansas City
- WSYX-TV (ABC) – Columbus
- KTNV-TV (ABC) – Las Vegas

August 8
- All News Channel

August 9
- WLS-TV (ABC) – Chicago
- KTRK-TV (ABC) – Houston
- KOMO-TV (ABC) – Seattle/Tacoma
- WRTV-TV (ABC) – Indianapolis
- WTNH-TV (ABC) – Hartford/New Haven
- WGGB-TV (ABC) – Springfield/Holyoke
- KOCO-TV (ABC) – Oklahoma City
- WPBF-TV (ABC) – West Palm Beach/Fort Myers
- WJRT-TV (ABC) – Flint/Saginaw
- KNXV-TV (ABC) – Phoenix
- WGNO-TV (ABC) – New Orleans
- WOKR-TV (ABC) – Rochester
- WROC-TV (CBS) – Rochester

August 10
- WRTV-TV (ABC) – Indianapolis
- WSOC-TV (ABC) – Charlotte
- KNXV-TV (ABC) – Phoenix
- WTAE-TV (ABC) – Pittsburgh

We continue to monitor coverage of this story and will provide you with ongoing updates. As stated in my 8/11 memo to you, we will proceed in using the drafted pitch letter to approach long-lead publications once we have your approval.

Please feel free to call me at (312) 856-8857 with any questions.

Kind regards.

W instock, Joel

From:
Sent: Friday, October 08, 1999 7:59 AM
T : david-elliott@uiowa.edu; joel-weinstock@uiowa.edu
Subject: Helminthic Parasites

I read a feature on the the CCFA web site about your recent clinical trial findings on Helminthic Parasites. Do you have more information available?

Also I would like to be find out about possible participation in any future trials.

Weinstock, Joel

Fr m:
Sent: Saturday, October 09, 1999 12:30 PM
T : Weinstock, Joel
Subject: crohns study

Dear Dr. Weinstock,
I had read that clinical studies with new drugs were limited to participants over 18 years of age, but I just read of a study on a drug for cystic fibrosis that had participants 13 to 17 years of age. Will your study with the helminth parasite use subjects under 18 years? We are still very interested in your research and optimistic now that we might be included in the study.

Sincerely.

Weinstock, Joel

From:
Sent: Wednesday, October 13, 1999 8:44 PM
To: joel-weinstock@uiowa.edu
Subject: IBD Worm Study

Dear Dr. Weinstock: I have read with much interest about your work with helminths and patients with IBD. I have an 11 y/o son with crohns, diagnosed this past June. Are you planning any satellite studies near the east coast? Are you planning any pediatric studies? I would be most interested in more information on your study and perhaps a place where I can read about it on the www. I have read only press releases at this point. Many, many thanks from a mother who would do anything to make her son better and keep him off as many drugs as possible.

Sincerely,

Weinstock, Joel

From: Michael Librarian Account [mlib@backup.vh.org]
Sent: Saturday, October 16, 1999 12:37 PM
To: joel-weinstock@uiowa.edu
Subject: VH Comment Form (fwd)

---------- Forwarded Message begins here ----------
From: web@vh.org
Date: Fri, 15 Oct 1999 20:27:55 -0500 (CDT)
To: librarian@vh.org
Subject: VH Comment Form

Document Last Referenced was :
http://www.vh.org/Patients/IHB/IntMed/Gastro/InflammatoryBowel.html

---

Input Field: Email
Response:
* rbvener1@gte.net

Input Field: Name
Response:

Input Field: Where Live
Response:
* Outside Iowa

Input Field: How would you describe yourself
Response:
* Patient

Input Field: What was your question
Response:
* Please tell me where I can participate in a study for ulcerative colitis involving Helminyhic
Therapy, as described here and in the Los Angeles Times newspaper. I would be willing to be a
participant in the first trials. I have had UC for 3 years and am currently taking asacol. Please
answer with the hospital which may be involved in this trial, possible UCLA, USC or any other
teaching hospital in California. I will go anywhere for a chance to participate.

Input Field: Patient age
Response:
* 61

Input Field: Patient gender
Response:
* Female

Input Field: Did you find the answer
Response:
* Found some information, but not all

Input Field: Where did you find your answer
Response:
* Gastrointestinal Section.

Input Field: Why did you look for an answer
Response:
* For my own learning

Input Field: What problems did you have using VH
Response:
* None, I could move around freely. Good site.

Input Field: Was valuable
Response:
* Yes

Input Field: Any additional comments
Response:
*

---------- Forwarded Message ends here ----------

Michael P. D'Alessandro, M.D.
Pediatric Radiologist, Associate Professor of Radiology, University of Iowa
Digital Librarian-In-Chief and Architect
Virtual Hospital/Virtual Children's Hospital/Virtual Naval Hospital
michael-dalessandro@uiowa.edu

W instock, Jo l

Fr m:
Sent: Thursday, October 14, 1999 8:53 AM
T : Weinstock, Joel
Subject: FW: ibd

Dr. Weinstock:

Do you have any additional information you can provide this gentleman?

Thank you.

----Original Message----

From:
Sent: Wednesday, October 13, 1999 11:51 PM
To: jeanne-mccabe@uiowa.edu
Subject: ibd

<A

HREF="<http://www.vh.org/Patients/IHB/IntMed/Gastro/InflammatoryBowel.html>">Vir tual Hospital: Iowa Health Book: Internal Medicine: Gastroenterology-Hepatol</ A> is there any more new news on this reasurch? it is difficult to wait for news when one of your children is affected by this illness.any new information would be aperatated.

thank you

W instock, Joel

From:
Sent: Tuesday, October 19, 1999 7:57 AM
T : Weinstock, Joel
Subject: RE: VH Comment Form (fwd)

Joel,

This was addressed to web@vh.org, was it sent to the original correspondent (rbvener1@gte.net)?

Thanks,

On Mon, 18 Oct 1999, Weinstock, Joel wrote:

> We appreciate your concern. We wish to help. We will keep your name on
> file and notify you when there will be a potential opportunity for you to
> participate. We promise to work as fast as possible and will not forget
> you.
>
> Joel Weinstock
>
> > ----Original Message----
> > From: Michael Librarian Account [SMTP:mlib@backup.vh.org]
> > Sent: Saturday, October 16, 1999 12:37 PM
> > To: joel-weinstock@uiowa.edu
> > Subject: VH Comment Form (fwd)
> >
> > ---------- Forwarded Message begins here ----------
> > From: web@vh.org
> > Date: Fri, 15 Oct 1999 20:27:55 -0500 (CDT)
> > To: librarian@vh.org
> > Subject: VH Comment Form
> >
> > Document Last Referenced was :
> > http://www.vh.org/Patients/IHB/IntMed/Gastro/InflammatoryBowel.html
> > ---------------------------------------
> > Input Field: Email
> > Response:
> >
> > ---------------------------------------
> > Input Field: Name
> > Response:
> >
> > ---------------------------------------
> > Input Field: Where Live
> > Response:
> > * Outside Iowa
> > ---------------------------------------
> > Input Field: How would you describe yourself
> > Response:
> > * Patient
> > ---------------------------------------
> > Input Field: What was your question
> > Response:
> > * Please tell me where I can participate in a study for ulcerative
> > colitis
> > involving Helminyhic
> > Therapy, as described here and in the Los Angeles Times newspaper. I

W instock, Jo l

From:
Sent: Wednesday, October 20, 1999 9:48 PM
To: joel-weinstock@uiowa.edu
Subject: Article in Science News August 14, 1999

Dear Mr. Weinstock:

I am a 33 year old woman who has had ulcerative colitis for about 15 years. I have been very lucky in that I have only had several major flares. I am very interested in your new research with the eggs of the whip-worm. Could you tell me where I could find out more, or if you have had any success with UC. I am unable to take almost any of the drugs currently used to treat the condition. Thank you very much.

Yours Truly,

Weinstock, Jo I

From: Summers, Robert
Sent: Monday, August 09, 1999 2:50 PM
To: Weinstock, Joel
Subject: Local reaction without asking for anything

This is a patient of mine with you guessed it, Crohn's disease. She is a nurse and a good observer. It is an observation that we shouldn't forget.

Congratulations Dr. Summers on your IBD findings!!! I think you are on to something really big. I now know 6 members of our family(on my fathers side)- who have IBD's and will tell them all!!!!

I will share a secret. Although I am doing very well with my Crohns, last year about this time, my 6 year old daughter contracted pinworms. Of course the rest of the family did, and by the time we discovered it, we had probably all been infested for 4-8 weeks. I remember at the time that I felt so good!!! No bloated feelings, no occassional "twanger pain", it was quite obvious!!
Anyway, thought I'd just boost your theory!!!
Blessings to you as you "research".
Diane

Carol D
8/9

Moore, Tom

From: Michael Librarian Account [mlib@backup.vh.org]
Sent: Friday, September 10, 1999 9:45 AM
To:
Cc: chad-rubak@uiowa.edu; thomas-moore@uiowa.edu; david-pedersen@uiowa.edu
Subject: VH Comment Form (fwd)

Mel,

Here is the information you are looking for:

http://www.uihealthcare.com/NewsEvents/News/1999/08/Worms.html

Michael
---------- Forwarded Message begins here ----------
From: web@vh.org
Date: Fri, 10 Sep 1999 00:12:32 -0500 (CDT)
To: librarian@vh.org
Subject: VH Comment Form

Document Last Referenced was :
http://www.vh.org/Welcome/UIHC/UIHCPhysDirectory/GastroHepMDs/JoelWeinstock.html
---
Input Field: Email
Response:

Input Field: Name
Response:

Input Field: Where Live
Response:
* Outside Iowa

Input Field: How would you describe yourself
Response:
* Allied Health Care Professional

Input Field: What was your question
Response:
* I read a squib published in the Montreal, Canada GAZETTE referring to an article published by Joel
Weinstock of the U. of Iowa in which he is reported to be doing research with intestinal worms for
the treatment of Crohn_s disease...I am interested in reading about his research and all follow-ups
as they occur.....as well as knowing about his resources!

Input Field: Patient age
Response:
* 27

Input Field: Patient gender
Response:
* Female

Input Field: Did you find the answer
Response:
* No

Response:
* GI Section

Input Field: Why did you look for an answer
Response:
* For my own learning

Input Field: What problems did you have using VH
Response:
*

Input Field: Was valuable
Response:
* Yes

Input Field: Any additional comments
Response:
*

---------- Forwarded Message ends here ----------

Michael P. D'Alessandro, M.D.
Pediatric Radiologist, Associate Professor of Radiology, University of Iowa
Digital Librarian-In-Chief and Architect
Virtual Hospital/Virtual Children's Hospital/Virtual Naval Hospital
michael-dalessandro@uiowa.edu

Moore, Tom

From: Michael Librarian Account [mlib@backup.vh.org]
Sent: Monday, September 20, 1999 12:44 PM
T :
Cc: chad-ruback@uiowa.edu; thomas-moore@uiowa.edu; david-pedersen@uiowa.edu
Subject: VH Comment Form (fwd)

Krissy,

Look at this article for the information you are seeking:

http://www.uihealthcare.com/NewsEvents/News/1999/08/08-09-1999Worms.html

Thanks,

Michael
---------- Forwarded Message begins here ----------
From: web@vh.org
Date: Fri, 17 Sep 1999 18:49:20 -0500 (CDT)
To: librarian@vh.org
Subject: VH Comment Form

Document Last Referenced was :
http://www.vh.org/Patients/IHB/IntMed/Gastro/InflammatoryBowel.html
---
Input Field: Email
Response:

Input Field: Name
Response:
* Krissy

Input Field: Where Live
Response:
* Outside Iowa

Input Field: How would you describe yourself
Response:
* Patient

Input Field: What was your question
Response:
* I was looking for the study of the helminths and their use for IBD. I was wondering if they were
looking for other people to be part of their study.

Input Field: Patient age
Response:
* 24

Input Field: Patient gender
Response:
* Female

Input Field: Did you find the answer
Response:
* Found some information, but not all

Input Field: Where did you find your answer

Input Field: Where did you find y . answer
Response:
*

Input Field: Why did you look for an answer
Response:
* Other

Input Field: What problems did you have using VH
Response:
* minimally informative

Input Field: Was valuable
Response:
* No

Input Field: Any additional comments
Response:
*

---------- Forwarded Message ends here ----------

Michael P. D'Alessandro, M.D.
Pediatric Radiologist, Associate Professor of Radiology, University of Iowa
Digital Librarian-In-Chief and Architect
Virtual Hospital/Virtual Children's Hospital/Virtual Naval Hospital
michael-dalessandro@uiowa.edu

Moore, Tom

From: Michael Librarian Account [mlib@backup.vh.org]
Sent: Tuesday, September 21, 1999 11:32 AM
T : Shenscheut@t-online.de
Cc:
Subject: VH Comment Form (fwd)

Henrik,

Here is the information on research on ulcerative colitis that you are looking for:

http://www.uihealthcare.com/NewsEvents/News/1999/08/08-09-1999Worms.html

Thanks,

Michael
---------- Forwarded Message begins here ----------
From: web@vh.org
Date: Tue, 21 Sep 1999 05:14:30 -0500 (CDT)
To: librarian@vh.org
Subject: VH Comment Form

Document Last Referenced was : http://www.vh.org/Welcome/DeptsClin.html
---
Input Field: Email
Response:

Input Field: Name
Response:

Input Field: Where Live
Response:
* Outside US

Input Field: How would you describe yourself
Response:
* Family Member or Friend of Patient

Input Field: What was your question
Response:
* My daughters illness for about 9 years is ulcerosa colitis. Today I found a information in a german
newspaper, that medical doctors of University of Iowa State started a new "worm-egg-therapy" to
regulate the overreaction of the immunsystem of patients with this illness. Please, forgive my bad
english, but I would be very interested, to get further information about this therapy and about
the way, to work with it. English is not a problem, because my daughter was a student of Iowa-State
University some years ago.
I would be very thankfull for more information, or possibly adresses and other assistence regarding
this matter.

Regards

Moore, Tom

From: Michael Librarian Account [mlib@backup.vh.org]
Sent: Wednesday, September 29, 1999 7:33 AM
To:
Cc: chad-ruback@uiowa.edu; thomas-moore@uiowa.edu; david-pedersen@uiowa.edu
Subject: VH Comment Form (fwd)

James,

Look here:

http://www.uihealthcare.com/NewsEvents/News/1999/08/08-09-1999Worms.html

Michael
---------- Forwarded Message begins here ----------
From: web@vh.org
Date: Tue, 28 Sep 1999 15:25:42 -0500 (CDT)
To: librarian@vh.org
Subject: VH Comment Form

Document Last Referenced was : http://www.vh.org/

Input Field: Email
Response:

Input Field: Name
Response:

Input Field: Where Live
Response:
* Outside Iowa

Input Field: How would you describe yourself
Response:
* Student

Input Field: What was your question
Response:
* I would like to get a copy of Dr. Joel Weinstock_s paper regarding IBD and parasitic helminths.

Thank you

Input Field: Patient age
Response:
*

Input Field: Where did you find your answer
Response:
*

Input Field: Why did you look for an answer
Response:
* For my own learning

Input Field: What problems did you have using VH
Response:
*

Input Field: Any additional comm s
Response:

---------- Forwarded Message ends here ----------

Michael P. D'Alessandro, M.D.
Pediatric Radiologist, Associate Professor of Radiology, University of Iowa
Digital Librarian-In-Chief and Architect
Virtual Hospital/Virtual Children's Hospital/Virtual Naval Hospital
michael-dalessandro@uiowa.edu

Moore, Tom

From: michael-dalessandro@uiowa.edu
Sent: Friday, October 15, 1999 8:27 AM
T :
Cc:
Subject: VH Comment Form (fwd)

Ruby,

Here is the information you are seeking:

http://www.uihealthcare.com/NewsEvents/News/1999/08/08-09-1999Worms.html

Michael

>Date: Thu, 14 Oct 1999 07:58:32 -0500
>From: "Michael Librarian Account" <mlib@backup.vh.org>
>Reply-To: "Michael Librarian Account" <mlib@backup.vh.org>
>To: michael-dalessandro@uiowa.edu
>Subject: VH Comment Form (fwd)
>Status:
>
>---------- Forwarded Message begins here ----------
>From: web@vh.org
>Date: Wed, 13 Oct 1999 21:55:26 -0500 (CDT)
>To: librarian@vh.org
>Subject: VH Comment Form
>
>Document Last Referenced was :
>http://www.vh.org/Providers/ClinRef/FPHandbook/Chapter04/16-4.html
>----------
>Input Field: Email
>Response:
>
>----------
>Input Field: Name
>Response:
>
>----------
>Input Field: Where Live
>Response:
> * Outside US
>----------
>Input Field: How would you describe yourself
>Response:
> * Family Member or Friend of Patient
>----------
>Input Field: What was your question
>Response:
> * Have you info on the research being done by Dr. Joel Weinstock with
>regards
>to Crohn_s and other
>intestinal diseases? Thanks.
>----------
>Input Field: Patient age
>Response:
> * 34
>----------
>Input Field: Patient gender

>Response:
> * Male
>----------
>Input Field: Did you find the answer
>Response:
> * No
>----------
>Input Field: Where did you find your answer
>Response:
> *
>----------
>Input Field: Why did you look for an answer
>Response:
> * Curiosity
>----------
>Input Field: What problems did you have using VH
>Response:
> *
>----------
>Input Field: Was valuable
>Response:
> * Somewhat valuable
>----------
>Input Field: Any additional comments
>Response:
> *
>----------
>---------- Forwarded Message ends here ----------
>
>----------
>Michael P. D'Alessandro, M.D.
>Pediatric Radiologist, Associate Professor of Radiology, University of Iowa
>Digital Librarian-In-Chief and Architect
> Virtual Hospital/Virtual Children's Hospital/Virtual Naval Hospital
>
>michael-dalessandro@uiowa.edu
>----------
>

Moor , Tom

From: Michael Librarian Account [mlib@backup.vh.org]
Sent: Monday, September 20, 1999 12:37 PM
T :
Cc: chad-ruback@uiowa.edu; thomas-moore@uiowa.edu; david-pedersen@uiowa.edu
Subject: Re: article in paper

Sherree,

Here is an article on the information you are interested in:

http://www.uihealthcare.com/NewsEvents/News/1999/08/08-09-1999Worms.html

Thanks,

Michael

> I'm not sure if you can help me but maybe you can let me know who to
> contact. I live in Louisville, Kentucky and in our newspaper there was an
> article about a study Dr. Joel Weinstock was doing with people suffering
> with chronic inflammatory bowel disease-crohns. I would like any
> information I can get about this study. My mother has crohns and has tried
> everything but with no results. It seems like her attacks are coming closer
> together and each time it takes her longer to recover.
>
> I would appreciate any help that you can give me.
> Thank you

Michael P. D'Alessandro, M.D.
Pediatric Radiologist, Associate Professor of Radiology, University of Iowa
Digital Librarian-In-Chief and Architect
Virtual Hospital/Virtual Children's Hospital/Virtual Naval Hospital
michael-dalessandro@uiowa.edu

Weinstock, Joel

From: stephen leigh [stephen.l@cwcom.net]
Sent: Friday, August 06, 1999 4:50 AM
To: joel-weinstock@uiowa.edu; weinstockj@mail.medicine.uiowa.edu
Subject: Intestinal worms: National Geographic

Dear Joel,

I am researching a new story for National Geographic TV about the use of live animals in medical treatments.

So far our story consists of a look at using maggots to clean necrotic tissue as well as the use of leeches in plastic surgery. However, I found an article in yesterday's Times newspaper (London) that told of your research on the use of intestinal worms as a treatment for IB sufferers. I have many questions I would like to ask you about you work and would appreciate the opportunity to talk to you. Perhaps I could give you a call later today (early morning for you as I am based in London)?

I would like to know:
1. How long you have been doing this research?
2. Is it something we could get TV footage of?
3. Would you be happy to interviewed about it?
4. How do the patients feel when you give them the worm eggs?
5. Is it an effective weight loss method?
6. Is the a small chance that the worms might survive and carry on reproducing?
7. What would your availability be like if we did ask to spend some time filming with you?
8. Is there any footage of a worm in situ that we could use?

If you get time, I would greatly appreciate a reply to this email.

I can be contacted on:
T: +44 207 292 1008
F +44 207 287 9941
or by replying to this email (my producer's address).

I look forward to hearing from you,
Kind regards,
Martin Williams
National Geographic TV

W instock, Joel

From: Kulig, Nanci [nanci.kulig@springnet.com]
Sent: Monday, September 13, 1999 1:44 PM
To: joel-weinstock@uiowa.edu; weinstockj@mail.medicine.uiowa.edu
Subject: your study results


NANCIK~1.VCF

Hi Dr. Weinstock.

I'm an editor at Nursing99, a professional journal aimed at nurses. I'm interested in writing a news piece about your preliminary findings concerning the use of parasitic worms for inflammatory bowel disease.

Could you tell me where I might find more information about your study? I read about it in a New York Times article, published on Tuesday, August 31, but the article didn't say where your findings have been published or if you have plans to publish.

I hope to hear from you soon.

Sincerely,

Nanci Kulig
Senior associate editor
Nursing99
1111 Bethlehem Pike
PO Box 908
Springhouse, PA 19477-0908
PH: 800-346-7844, ext. 1309
FAX: 215-653-0826
Email: nanci.kulig@springnet.com

Nanci Kulig
senior associate editor
Nursing99
1111 Bethlehem Pike
PO Box 908
Springhouse, PA 19477-0908
PH: 800-346-7844, ext. 1309
FAX: 215-653-0826
Email: nanci.kulig@springnet.com

Weinstock, Joel

From: Nicolle Charbonneau [ncharb@interlog.com]
Sent: Monday, August 09, 1999 11:26 AM
To: joel-weinstock@uiowa.edu
Subject: Media query re: article in Aug. 7/99 New Scientist

9:20 a.m. EDT
August 9, 1999
Toronto, Canada

Dear Dr. Weinstock.

My name is Nicolle Charbonneau, and I'm a reporter with a publication called HealthScout, an online health news service. I'm writing in regards to your article on parasitic worms and inflammatory bowel disease, which was written about in the Aug. 7 issue of New Scientist. I'm going to be writing an article about your work for our website, and I was wondering--would you be available for a brief phone interview today?

I can keep my questions brief, since you're probably rather busy, but I find that it always helps to go straight to the source with questions, and to be honest, it would reassure me that I understand things accurately.

I'll give you a call in case you don't have a chance to check your e-mail. I hope we can arrange a time to chat--my phone number and e-mail address are at the end of this message. I'm looking forward to speaking with you!

Warm regards,

Nicolle

Nicolle Charbonneau
Reporter
HealthScout
www.healthscout.com <http://www.healthscout.com>
Tel./Fax (416) 406-3907
ncharb@interlog.com <mailto:ncharb@interlog.com>

South Africa ✓ BSC
Interviews
Done

Weinstock, Joel

From: David Lyerly [dlyerly@techlabinc.com]
Sent: Monday, August 16, 1999 8:36 AM
To: joel-weinstock@uiowa.edu

Dear Dr. Weinstock,

My name is David Lyerly and I am Vice-President of TechLab, Inc., a small diagnostic/biotechnology company located in Blacksburg, VA. Our company was founded by scientists from the Anaerobe Laboratory of Virginia Tech. As you might suspect from our background, TechLab focuses on microbiology of the intestine, with particular emphasis on areas such as diagnostics for enteric diseases (C. difficile, Giardia, Entamoeba, Cryptosporidium) and the effects of various artificial food additives (artificial fats, sweeteners) on the intestinal flora. In addition, we are funded by NIH for research on Entamoeba histolytica, a project that we have ongoing with the University of Virginia. In addition to these projects, we also have a collaborative project with UVA on the detection of lactoferrin as a marker for fecal leukocytes. This work is based on the work of Dr. Richard Guerrant (Div. Geographic Medicine, UVA) on ways to detect inflammation in the intestine, and we have been working with Dr. Guerrant for a number of years in this area. We market a test for human lactoferrin in stool specimens, and it works quite well as a screen for inflammatory diarrheas and for measuring the high levels of lactoferrin in IBD patients.

The reason that I am contacting you is because of an article which appeared last Sunday in our local newspaper, the Roanoke World News and Times. The article, entitled "Researcher says worms may fight bowel disease", focused of course on your work using Ascaris to treat persons with inflammatory bowel disease. I found the article to be extremely interesting and I think your research would be of interest to readers of our "Diarrhea Digest", a newsletter that TechLab sends to about 2500 clinical labs in the U.S. and around the world. I could use the material that was published in the article, but because we stress the science that goes into the newsletter articles, I wanted to check with you and see if your work on this topic had been published. Alternatively, would you be interested in writing a 2-3 page article on your work for our newsletter? We would pay you $250 for the article and of course, you would receive full credit for it. I think such an article would receive much interest and if you are willing to submit it, I would like to put it in our Summer newsletter as the feature article. I am putting this issue together now and hope to have it completed within the next couple of weeks.

I would be happy to send you a copy of the newspaper article as well as our latest addition of Diarrhea Digest for your perusal. Thanks for allowing me to tell you a little about TechLab and I appreciate your time and consideration for my request.

Best wishes,

David M. Lyerly, Ph.D.
Vice-President, TechLab, Inc.
1861 Pratt Drive, Corporate Research Center
Blacksburg, VA 24060-6364
Phone 1-800 TechLab
FAX 540-953-1665
e-mail: dlyerly@techlabinc.com

Weinstock, Jo I

From:
Sent: Saturday, October 30, 1999 7:00 PM
To: joel-weinstock@uiowa.edu
Subject: Crohns disease

All health professionals are very busy so I will keep this e-mail very short.

I am a general practitioner in Adelaide, Australia and I have recently come across your article in New Scientist about "worms" and Crohns disease. Besides having patients with this disease I am also a sufferer and at present on Prednisolone 25mg daily.

I suspect you have been inundated with requests for information about your research however I would appreciate an update on further clinical success or otherwise.

Thankyou

DR JOEL WEINSTOCK
UNIVERSITY OF IOWA
FAX # 319 353 6399
OCT 24TH, 1997 - 9:15 AM

ATTENTION = CLAUDIA

THANK YOU FOR SENDING THE DOCUMENTS, OF HIGH INTEREST TO ME.

I HAVE A VERY STRONG IMMUNOLOGY PROTECTION AND I NOW BELIEVE I FOUND AN EXPLANATION TO THE PROBLEMS I AM FACING CONCERNING MY POOR REGULATION OF BOWEL BEHAVIOUR (EXCESSIVE REACTION TO FOODS OF ALL KINDS, SOFT FECES, ENERGY LEVEL NOT ALWAYS AT NORMAL) -

PLEASE KEEP ME UP-DATED, SINCE I WISH TO TEST THE TREATMENT AS SOON AS IT IS APPROVED -

THANK YOU FOR YOUR KIND ASSISTANCE.

WARMEST REGARDS

Weinstock, Jo I

Sent: Friday, October 29, 1999 10:17 PM
To: joel-weinstock@uiowa.edu
Subject: Crohn's Disease and Worm Treatments

Dr. Weinstock:

I recently read an article in The Houston Chronicle outlining the success of your research with IBD and Crohn's. I have been fighting Crohn's for 10 years now and am very interrested in participating in your study. I live in Dallas, TX and would be willing to do whatever neccessary to recieve a treatment of the worm eggs.

I was just recently released from the hospital suffering from a partial obstruction. We have been trying to put-off having surgury in hopes that a cure was in the pipline. Your research is the most promising thing I have heard of. I have tried Remicade, 6MP, Asocol, and am controlling disease with Prednisone.

I would be most grateful if you would allow me to participate.

Thanks for your efforts.

Sincerely,

Weinstock, Jo I

Sent: Wednesday, October 27, 1999 6:03 AM
To: joel-weinstock@uiowa.edu; weinstock@mail.medicine.uiowa.edu
Subject: HELMINTH TREATMENT

Dear Professor Weinstock:

My daughter has Chron's Disease for already 12 years. She underwent 2 surgical interventions. These days, she does not seem to respond to any of the drugs she is taking. I am trying to find any alternative/supplement to conventional drugs. Your research on helminth tretament caught my attention. I belive in the theory that the GI tract has to be challenged by exposure to intestibnal parasites (or pathogens). That, in combination with genetic make up (my wife is an Ashkenasi Jew, I am a sepharadic Jew) could explain my daughter's illness.

Are you pursuing your research on helminth parasites. If so, please let me know about the status of your research. Any publications? Is there any way she could participate in the research in collaboration with her gastroenterologist in Atlanta? Any word of wisdom would be helpful.
Thank you for taking the time to read this e-mail from a confused and desperate father.

==========

Weinstock, Jo I

To:
Subject: RE: VH Comment Form (fwd)

Thank you for expressing interest in our research. I am sorry to hear you have IBD. We are working as fast as possible to determine the possible therapeutic potential of this new treatment. We can not at this time enter you in the study because of geographical considerations. We will keep your name on file and consider you for entry when the study expands.

Joel Weinstock

Sent: Tuesday, October 26, 1999 8:39 AM
To: joel-weinstock@uiowa.edu
Subject: Re: VH Comment Form (fwd)

> > Input Field: What was your question
> > Response:
> > * I read an article that featured Joel V. Weinstock, M.D. re: his
> > "worms" -
> > it said he would be
> > conducting another study in January. How can I participate in this study.
> > I
> > live in Houston, TX
> > and have Ulcerative Colitis (my doctor is Ronald M. Rance M.D. - Houston).
> >
> > Could I receive my
> > "medication" via mail and return any required results through the mail
> > -or- my
> > doctor ??
> > Thank you for your consideration,
> >
> > ---
> > Input Field: Patient age
> > Response:
> > * 55
> > ---
> > Input Field: Patient gender
> > Response:
> > * Male
> > ---
> > Input Field: Did you find the answer
> > Response:
> > * No
> > ---
> > Input Field: Where did you find your answer
> > Response:
> > * I read an article in the Houston Chronicle Oct. 18, 1999 about Joel
> > V.
> > Weinstock, M.D. and his
> > "worms" discovery.
> > ---
> > Input Field: Why did you look for an answer
> > Response:
> > * For taking care of a patient
> > ---
> > Input Field: What problems did you have using VH
> > Response:
> > * None - Great Site
> > ---
> > Input Field: Was valuable
> > Response:
> > * Somewhat valuable

> > ---------- ----------
> > Input Field: Any additional comments
> > Response:
> > * Please consider allowing me to participate in the study. I have had
> > Ulcerative Colitis since 1993
> > and have taken Prednisone until it has deteriorated my bones to the point
> > I now
> > need a hip
> > replacement.
> > ----------
> > ---------- Forwarded Message ends here ----------
> >
> > ----------
> > --
> > Michael P. D'Alessandro, M.D.
> > Pediatric Radiologist, Associate Professor of Radiology, University of
> > Iowa
> > Digital Librarian-In-Chief and Architect
> > Virtual Hospital/Virtual Children's Hospital/Virtual Naval Hospital
> >
> > michael-dalessandro@uiowa.edu
> > ----------

Michael P. D'Alessandro, M.D.
Pediatric Radiologist, Associate Professor of Radiology, University of Iowa
Digital Librarian-In-Chief and Architect
Virtual Hospital/Virtual Children's Hospital/Virtual Naval Hospital
michael-dalessandro@uiowa.edu

Weinstock, Joel

Sent: Wednesday, October 20, 1999 9:48 PM
To: joel-weinstock@uiowa.edu
Subject: Article in Science News August 14, 1999

Dear Mr. Weinstock:

I am a 33 year old woman who has had ulcerative colitis for about 15 years. I have been very lucky in that I have only had several major flares. I am very interested in your new research with the eggs of the whip-worm. Could you tell me where I could find out more, or if you have had any success with UC. I am unable to take almost any of the drugs currently used to treat the condition. Thank you very much.

Yours Truly,

Weinstock, Joel

Sent: Wednesday, October 20, 1999 12:42 PM
To: Joel-Weinstock@uiowa.edu
Subject: Address

Sir
I meant to give you our address for your file in case my son would be able to participate in your worm study for IBD. It is

Weinstock, Joel

Sent: Monday, October 25, 1999 2:21 AM
To: joel-weinstock
Subject: Parasites - IBD

I read in the paper today an article on how you have researched using "regular doses of worms" for treating people with inflammatory bowel disease.

I have colitis and am currently on cyclosporine after being resisitant to steriods and other medications normally used to control colitis.

Is there any possible way I could partake in the study?
I live in Edmonton, Alberta, Canada.

I am desperate to try hang on to my colon!
I was diagnosed at age 30 - guess I'm a late bloomer.
All my life I have been in excellent health until I came down with colitis last November.
I was origally taking 5-ASA, however crashed mid-may, spent weeks on high doses of IV prednosone.
My health continued to fail. At this point I weighed 100 pounds and ended up in the hospital for 4 weeks with a PICC.
After almost losing my colon to surgery, my specialist, knowing my persistant personality decided to try the cyclosporine.
I was a miracle! After one month I was released from the hospital 10 pounds heavier.

Since then my health has been much better, however I'm still not 100%.
I have gained an additional 5 pounds (my total weight lose was 30 pounds, I'm 5'9").
I have been on cyclosporine since June and realize I cannot be on this medication for a long period of time.
In addition I am recently engaged and have high hopes of becoming drug free and starting a family.
I live in fear once I am taken off the cyclosporine, I will once again be faced with the team of surgeons looking for my colon!

I realize that I do not live in Iowa, however as I mentioned I am desparate to find help for this disease.
Perhaps my specialist here in Edmonton could monitor me? Perhaps I could travel to Iowa?
I'm more than willing to keep a very detailed diary or whatever may be required.

I just want to be drug free and have all my organs!

Please let me know if there is any way I can be part of your clinical trial.
Even if I am not cured, I feel it is important to try any therapy that may help colitis.
I would do anything to prevent someone else from having to live with this condition.

Thank you for your time.

Weinstock, Joel

Sent: Wednesday, October 20, 1999 3:48 PM
To: joel-weinstock@uiowa.edu
Subject: Intestinal parasites

Hi, my name is read an article on your research in the news paper. For once in the 10yrs I've had Irritable bowl, I've final feel there is nope.
I have been suffering with this condition since I was 21, I've had surgery's to remove the scare tissue and have spent many nights in the emergency room, just to be told I have Irritable bowls. I am writing to you because I need help. I can not live this way anymore, I have a wonderful family who supports me and never says anything to me when we have to cancel a trip or just going to a ball game, because "mom's sick". I have been put on many medications that haven't helped, only doped me up. I've been to herbal therapy, tried colonics, and had 4 surgery's, only to be let down once again. I am a very strong person and I just can't take the pain anymore. I stop eating so that I don't have to diarrhea and pain. I have dropped from 140 to 103 pounds. As I sit here writing this I can't help but cry. I love my kids and my husband with all my heart, but am fighting with the question of what to do. should I save them from a life with me or stay and be there for them when they are going on there first date or just been dumped by their boyfriend. I am telling you things that I've never told anyone and I don't know why. I guess when I read the article on you , I got my hopes up that someone out there cares. The doctors I've seen just keep telling me not much is known about it and what can be done for it. Please let me know what I can do, you know I must be desperate ..... I'm willing to drink worm eggs!!! Ha Ha. Thanks for your time.

Keep up the great work,

Weinstock, Joel

Sent: Friday, October 22, 1999 9:48 AM
T : joel-weinstock@uiowa.edu
Subject: Helminths cure(!)

Dear Dr. Weinstock,

I read with great interest the article in the NY Times about the Helminths treatment. It makes logical sense.

I do not know how to convey my desperatness. Perhaps the following will: I am a vegetarian, but I am willing to swallow the worm eggs.

I have steroid dependent Crohn's since over four years WITHOUT remission. I have tried Interleukin-10 but the dose given was not effective. I do not think Asacol is doing much but I continue to take it. I have tried homeopathic and Ayurvedic medicines. Now I am on Budesonide but I do not see much improvement in my condition. I do not feel like eating, have lost weight and the lack of nutrition must be affecting the rest of my body. Our lives have been at a standstill.

I am very interested in the open label treatment. Although I am in Connecticut, I am willing to do what is necessary to try it. Please HELP!

Weinstock, Joel

Fr m:
Sent: Thursday, October 21, 1999 9:36 PM
To: joel-weinstock@uiowa.edu
Subject: intestinal worms to treat inflammatory bowel disease study

I have been stuck with Crohns since 1994 and am interested in participating in a study.

In the story at:
http://www.uihealthcare.com/NewsEvents/News/1999/08/08-09-1999Worms.html

I read that "They are now organizing a double-blind, clinical trial with additional patients to further test the benefit of this unique treatment. "

How do I get involved with the clinical trial?

I live in Minneapolis, MN and am willing to travel to a couple of times a month.
I have had Crohns disease since 1994.

If you need anymore info, please let me know.

Weinstock, Jo I

Sent: Tuesday, October 05, 1999 8:47 PM
To: weinstockj@mail.medicine.uiowa.edu
Subject: clinical trials

Dear Dr. Weinstock,

I recently read an article in the Los Angeles Time's about your research at the University of Iowa in autoimmune diseases and of your clinical study involving patients with chronic inflammatory bowel disease.

From that article I used the web and U of I's virtual hospital site to read your research article on 'Helminthic Therapy of Inflammatory Bowel Disease' which concludes with the statement that you would be organizing additional clinical trials.

The reason I am writing is to find out if their will be any collaborative studies or trials in the Los Angeles area. I would be interested in participating in such a study. Any information you could forward would be deeply appreciated.

Weinstock, Joel

Sent: Tuesday, October 05, 1999 4:54 PM
To: joel-weinstock@uiowa.edu
Subject: helminth research

Dr. Weinstock:

I am extremely interested in your research on the treatment of Crohn's disease with helminth worms.

My 19 year old daughter (Vanessa) has Crohn's disease. She has just gone through her second operation removing diseased intestine tissue. In her first operation about 2 1/2 years ago, approx 3 feet of small intestine was removed. Her operation six weeks removed another 9 inches of small intestine and about half of her large intestine. She has been on steroid treatments (sorry about my spelling) Prednasone. She has taken daily dosages of Asicol. She has tried two Remacade treatments and a short treatment of Immuran. None seem to work, and we are extremely worried that because of the natrue of Crohnes, that she will have more surgeries. As you can see, she doesn't have much time left before the quality of her life will suffer.

The article on your research (War on Germs is Enough to Make You Sick -- LA Times) was extremely interesting. I think that it is quite possible that you have found the cause of her Crohnes. Everything fits. I just hope that you have also found the cure.

What can I do to learn more about what you are doing? What can I do to get my daughter involved in your research?

Vanessa is currently a student at California Polytechnic University - San Luis Obispo (had to drop out last quarter due to the surgery but will be back in January). Cal Poly is about a 5 hour drive from UC Davis, and about a 3 hour drive from UCLA / USC. We live just outside Sacrametno (next to Davis). Arrangements could be made for her to travel to the University of Iowa, or any other location that you are doing research (the article said that you were trying to set up a nationwide study).

I believe that her Crohnes was triggered by an Amoeba that she carried for quite some time before it was discovered and taken care of. I believe that your research fits in perfectly with her reaction and disease. It makes absolute sense to me that her body is still trying to fight off the Amoeba, and that your worms could take its place and be a cure. I believe that the current medical profession does not have the answer with drugs and surgery. I believe that you have the key. Please take the time to help her.

Please call or write

Weinstock, Joel

Sent: Thursday, September 09, 1999 4:15 PM
To: joel-weinstock@uiowa.edu
Subject: worms

hi

i heard you on the BBC talking about your research for treating ilitis colitis.

i am from BAHRAIN and i have IC.

i would like to find out as much information as possible about this treatment and any other research or treatment that you might have for this disease.

could you kindly send me ithis info ?

best regards

W instock, Joel

Sent: Tuesday, September 14, 1999 8:43 AM
To: joel-weinstock@uiowa.edu; David-Elliott@uiowa.edu; Robert-Summers@uiowa.edu; Khurram-Qadir@uiowa.edu
Subject: HEllo

. I am an ulcerative colitis patient who read with much interest an article about your researcn on Helminthic therapies that was posted on the ccfa website. I am unfortunately very far away from Iowa but am willing and hoping that you have open space in your upcoming expanded trial of the new therapy and if that is so that some method may be reached whereby I could particpate. I have had colitis for over 2 and a half years (am 24 years old) and have been on doses of predinsone for much of that time. I am understandably eager to get off the drug. I am currently on a relatively low dose, 10mg a day. Please respond at your earliest convenience and have a good day,

Weinstock, Joel

Sent: Tuesday, September 14, 1999 10:53 AM
T : joel-weinstock@uiowa.edu
Subject: RE: HEllo

i am by the way located in maryland. in case I neglected to mention that.

thanks yet again,

Weinstock, Jo I

Sent: Friday, September 17, 1999 10:56 AM
T : 'joel-weinstock@uiowa.edu'
Subject: Your Crohn's research

Dear Dr Weinstock:

I read the very interesting account of your 'worm research' on Crohn's and ulcerative colitis in the Aug 31 edition of the NY Times.

My 19-year old son Omar was diagnosed with both Crohn's and ulcerative colitis this past summer. The disease is limited to the colon (although the entire colon is affected).

The basic treatment plan initiated with daily 60 mg of prednisone which is being tapered back while bringing up asacol in the background. However, he has not tolerated asacol well and it is not clear at this point what a satisfactory non-steroid treatment will be.

Omar is a top student at the University of Virginia and just started his second year. He is strong in many areas, including math and science, and it occurred to me that he might be willing to provide you with additional test data, perhaps by working under faculty at UVA also specializing in research on inflammatory bowel disease.

While I have not yet discussed this with him (and am only now sending out the article to him), do you think the results are promising enough to engage other subjects in experimental treatment?

As a former Peace Corps volunteer and having worked in developing countries all my adult life, I have also been curious on occasion about parasitic relationships worms might have with humans! Your break-through research, as reported in the Times story, is highly interesting even without my own family situation. If you could refer me to a more complete account of your work and how the autoimmune thesis came about, I would be most grateful.

Sincerely,

Weinstock, Joel

Sent: Friday, September 17, 1999 10:44 PM
To: weinstockj@mail.medicine.uiowa.edu
Subject: Helminth larvae and Crohn's disease

Dr. Weinstock-

I recently read a brief review of your recent study involving helminth larvae. I am a biologist in Reno, NV. I have been suffering from Crohn's disease for nearly four years. I have had little success with traditional medications and have become increasingly frustrated. I realize that your study is preliminary and the sample size is small but I would be grateful to read anything you might have at your disposal on the subject.

I appologize to invade your privacy as I have, I am simply eager for alternative solutions. I am taking such large doses of medications that I sometimes wonder if I will ever be the same.

Respectfully yours

W instock, Joel

From:
Sent: Saturday, September 18, 1999 12:22 PM
To: 'joel-weinstock@uiowa.edu'
Subject: Colitis and worms

I read the recent New York Times article on a possible treatment for ulcerative colitis with great interest. I have suffered from UC for several years now, and would be interested in participating in any studies that might help further our understanding of and treatment for the disease. If you or your colleagues are conducting clinical trials in the future, I would appreciate the opportunity to participate.

Sincerely,

Weinstock, Jo I

From:
Sent: Sunday, October 03, 1999 10:26 PM
To: joel-weinstock@uiowa.edu
Subject: Crohn's Disease

As the parents of a 32 year old daughter with a severe case of Crohn's disease, my wife and I read with interest about your team's experimental use of helminths to treat Crohn's patients. Unfortunately, she has lost all but five feet of her small intestine. Fortunately, she is now under the care of Dr. Stefan Targon and his team at Cedars Sinai in Los Angeles.

We recognize that your work is experimental and in its early stages. However, we are always exploring developments in the field and would appreciate any information (including a copy of your presentation to the American Gastroenterological Association) about your work that you could provide to us.

Thanks.

Weinstock, Joel

Sent: Saturday, September 25, 1999 7:02 AM
T : joel-weinstock@uiowa.edu
Subject: Helminthic therapy of IBD

Dr. Weinstock,
I am a 39 year old male just diagnosed with ulcerative colitus. While browsing the CCFA homepage I came across the article you co-authored with Drs. Elliott, Summers, and Quadir. I find the premise of your research with helminthic parasites and immune system response very interesting, and the results of your preliminary research seem to be very promising. On behalf of myself and the millions of other UC sufferers, I encourage you and your associates to continue with this research and thank you for the hope that one day a cure may be found for this disease.
Sincerely,

Weinstock, Joel

Sent: Monday, September 27, 1999 4:06 PM
T : 'Weinstock, Joel'
Subject: RE: Colitis and worms

Thanks for your quick response. FYI: I noticed I wrote my e-mail address incorrectly below.

J

----Original Message----
From: Weinstock, Joel [mailto:joel-weinstock@uiowa.edu]
Sent: Saturday, September 18, 1999 11:42 AM

Subject: RE: Colitis and worms

I am sorry to hear of your disease. We are moving toward larger studies. We happily will keep your name on file and notify you when there will be an opportunity for you to participate. We hope to be of help in the near future.

Joel Weinstock

> ----Original Message----
> Sent: Saturday, September 18, 1999 12:22 PM
> To: 'joel-weinstock@uiowa.edu'
> Subject: Colitis and worms
>
> I read the recent New York Times article on a possible treatment for
> ulcerative colitis with great interest. I have suffered from UC for
> several
> years now, and would be interested in participating in any studies that
> might help further our understanding of and treatment for the disease. If
> you or your colleagues are conducting clinical trials in the future, I
> would
> appreciate the opportunity to participate.
>
> Sincerely,
>
>
>
>
>
>

Weinstock, Joel

Fr m:
Sent: Monday, September 27, 1999 11:56 PM
To: joel-weinstock@uiowa.edu
Subject: UC

Dear Dr. Weinstock,

I recently read about your work with parasites in treating ulcerative colitis. I am a second year medical student at Yale, and my fiancee is also a second year medical student at Albert Einstein. My fiancee was diagnosed with UC three years ago, and despite recurrent "tapers" of prednisone and several formualtions of 5-ASA drugs, he has not had even one day of relief or remission. I read that more trials of this study were going to take place. Will this be a multi-center trial? If so, are there centers in the New York or Connecticut area that will be participating? Is it feasible to think that he may be able to take part in this research? Your work in this feild has given us one more reason to hope that someday this disease will have, at the very least, the tools for effective management. For that, we thank you.

Sincerely,

****

Weinstock, Jo I

Fr m:
Sent: Monday, October 04, 1999 11:14 AM
To: joel-weinstock@uiowa.edu
Subject: Crohn's patient volunteer

Dear Dr. Weinstock,

As a Chrohn's patient, I am interested in your research with parasitic worms, with the hope of volunteering for your reasech treatment myself. Although I would guess you have many such requests, I would nevertheless like to give you a brief and rough medical history for your consideration. I am an otherwise healthy 36 y.o. male. First serious symptoms appeared in January 1999, confirmed by colonoscopy in February. Went into remission with about 60 days of Asacol and Prednisone (40 mg/day) but flared up again as Prednisone dosage was reduced. A treatment of Remicade and original daily dosage of Prednisone were effective for about another 60 days, but flared up again as Prednisone dosage was reduced. Another treatment of Remicade was effective in reducing symptoms, but I am back on 40 mg/day of Prednisone, as well as Asacol (2400 mg/day), Imuran (150 mg/day), and Metronidazol (1000 mg/day). My main concern is not being able to get off Prednisone, of secondary concern are the other medications.

My medical records of course are available. My time and costs are not an issue. My doctor will be attending the conference in Pheonix this month. I am available to go if it would be at all helpful.

Thank you, and I look forward to hearing from you.

Sincerely,

PS. If you can not include me in your research, can you refer me to someeone else doing similar research or to sources of information on the subject?

Weinstock, Joel

Fr m:
Sent: Monday, October 04, 1999 3:58 PM
T : Weinstock, Dr. Joel
Subject: Helminthic Therapy of Inflammatory Bowel Disease

Dear Dr. Weinstock:

I am a Clinical Professor of Medicine at the University of California San Francisco, a Board Certified Internist and a Fellow of the American College of Physicians. But who I really am is the concerned father of a 41 year old son who has had ulcerative colitis for at least one year which continues in an active phase despite all sorts of aggressive therapy, short of surgery. My son is under the care of an excellent gastroenterologist at UCSF, James Ostroff, M.D., but Dr. Ostroff has not yet heard about your research. I have taken on the task of learning as much about it as I can.

As you can imagine, I am interested in keeping current with the work that you and others are doing in the area of helminthic therapy of inflammatory bowel disese. I have read reports in the press, but would like to follow this area of research more closely. Any guidance that you you can give me will be greatly appreciated.

Yours sincerely,

Weinstock, Jo I

From:
Sent: Wednesday, October 20, 1999 11:52 AM
To: Joel-weinstock@uiowa.edu
Subject: Where can I get the worms??

Oct 20

Sir
I read about your research in the Cincinnati Enquirer this morning. My son who is a student at The Ohio State University was diagnosed with Crohn's disease at the age of 18 1/2 he is now 20. Last fall (1998) he lost 30 pounds in about five days and lost about 2/3 of his hair over the next few months, was in a great deal of pain and bleeding. He now has his disease under control but still has flare ups which are controlled with steroids and asacol. He seems to think his condition gets worse when he eats or drinks more sugar (which is also not as availble in poorer countries). As a child he had many ear infections for which he took antibiotics - maybe he doesn't have worms.
Your research seems logical and I would like for my son to ingest some worms. Could you tell me how to go about getting my son involved in your research or if anyone at The Ohio State University is duplicating your research?
Your help would be much appreciated!!

it to Print"

The New York Times

.... No. 51,631 Copyright © 1999 The New York Times TUESDAY, AUGUST 31, 1999

EXHIBIT "B"

In Pursuit of Autoimmune Worm Cure

By ANDY NEWMAN

For most of Western history, the average child walked around with a bellyful of parasitic worms: pinworms, tapeworms, hookworms. Then modern civilization came along, put shoes on the children's feet, installed sewers and stopped using human waste as fertilizer, and the worms mostly disappeared.

But there may be a downside to all this hygiene. Children in industrialized countries, which are relatively worm-free, have a much greater tendency than those in other countries to grow into adults with autoimmune disorders (in which the body is attacked by its own immune system), like rheumatoid arthritis, multiple sclerosis, lupus and inflammatory bowel disease.

Maybe this is a coincidence, but maybe not. Recently, researchers at the University of Iowa gave a drink containing the eggs of a half-inch-long parasitic worm to six people suffering from acute, chronic inflammatory bowel disease. Five went into remission, and the sixth improved substantially.

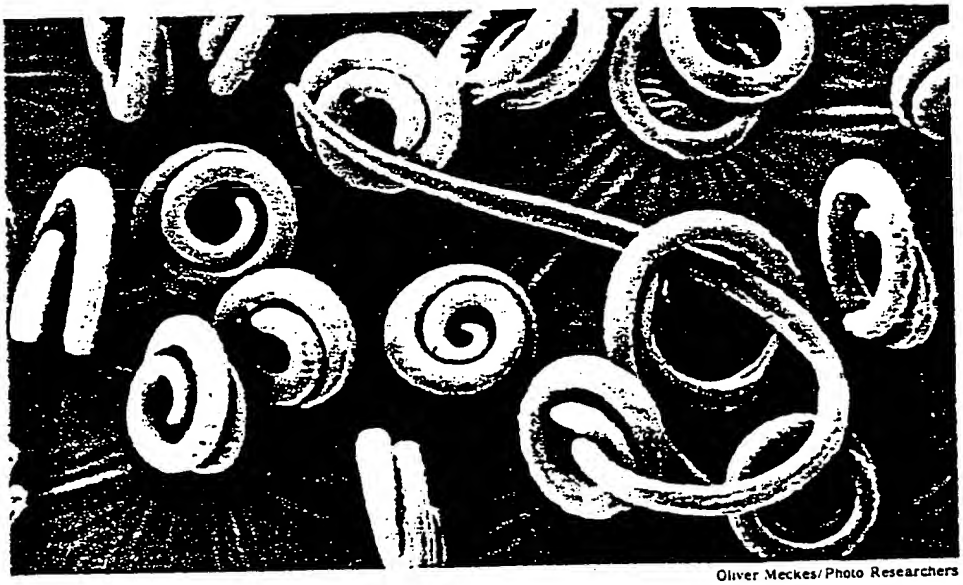
None got sick from the worms; all gradually relapsed after the worms left their systems. (For safety, the researchers used worms that normally live in pigs' intestines and were unable to reproduce and persist in their human hosts.) "Every one of those patients is begging to be re-treated," said the lead researcher, Dr. Joel Weinstock.

The sample was admittedly small. But the preliminary results, which made a big splash at an American Gastroenterological Association conference in May, could offer hope for the hundreds of thousands of Americans who suffer from inflammatory bowel diseases, mainly Crohn's disease and ulcerative colitis, which cause chronic and often bloody diarrhea, pain and weight loss.

"It's very interesting work," said Dr. Stephen Hanauer, co-director of the inflammatory bowel disease center at the University of Chicago School of Medicine and an international authority in the field, who was not connected with the study. "It needs to be substantiated in controlled trials, but there is some biologic sense to it."

Dr. Weinstock is one of a growing number of subscribers to what is known as the hygiene hypothesis — the idea that the war on germs and other contaminants is producing some unintended consequences.

A Swedish study in The Lancet, a medical journal, found this year that children of families that used antibiotics and vaccinations had more allergies than children of families that avoided them. And researchers in Israel found that rats raised in germ-free environments developed more arthritis and diabetes than other rats.



Oliver Meckes/Photo Researchers

Parasitic worms like these trichinella have been reviled and largely eliminated from human intestines, at least in the industrialized world. But they may have their uses.

Dr. Weinstock's team believes that Crohn's disease flares up when an immune system that has evolved to deal with multiple invaders finds itself unable to adapt to a more sterile environment.

Dr. Hanauer says the concept seems sound. "There are other potential explanations," he said. "But this is an interesting concept because it is holistic across the other immune diseases."

Dr. Weinstock got the idea for the worm cure three years ago while editing journal articles on parasites of the liver and intestine. In looking over decades of literature, he kept running across the idea that all well-adapted parasites perform some useful function for their hosts, if only to assure that the host survives to give them a home.

He and his colleagues began wondering about worms called helminths, which have been with humans for thousands of years. "We were racking our brains trying to think of what the benefits of these guys might be," he said. "Then the autoimmune disease hypothesis came up."

The hypothesis is based on the widely held theory that the immune system, when challenged by an invader, fights back with white blood cells of the Th1 or Th2 type. Th1 cells attack the body's infected cells, while Th2 cells go after dangerous microbes before they even invade the body's cells.

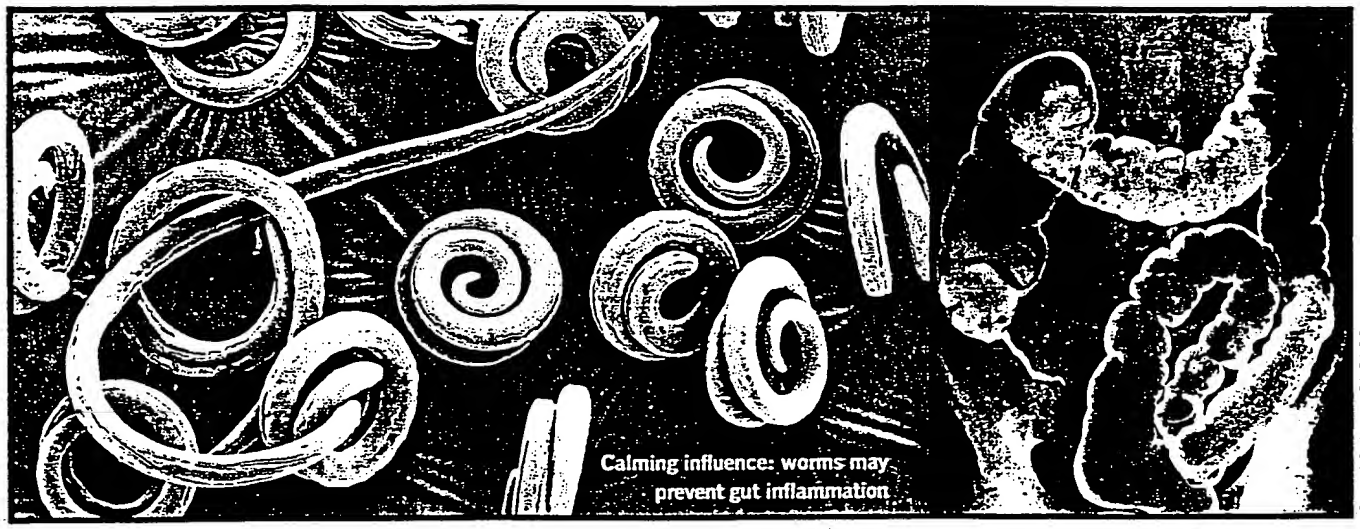
Dr. Weinstock thinks inflammatory bowel diseases develop when the body overreacts to the normal bacteria in the digestive tract, unleashing a salvo of Th1 cells that end up damaging the colon and bowel themselves. Helminths, he says, trigger a Th2 response, which dampens the Th1 response.

The helminth cure, if it proves effective, could have the side benefit of rehabilitating the reputations of parasitic worms, which have remained widely reviled even as some intestinal bacteria like acidophilus and lactobacillus have become popular over-the-counter supplements. The parasites, Dr. Weinstock said, have been victims of unfair if understandable prejudice. "Some worms do cause problems," he said, "but some cause very few problems."

On the other hand, he cautioned against ingesting worms without proper medical supervision. For this reason, he would not identify the precise species of helminth he uses in his research. "We don't want people going off to Mexico or who knows where else trying to expose themselves to helminths that are not controlled and getting who knows what kinds of consequences," he said. "If people run out and get hurt, I'm not going to feel really good about this."

Wonderful worms

Far from being harmful, a few gut parasites could do you the world of good



Calming influence: worms may prevent gut inflammation

Eye of Science/CNRI/SPL

REGULAR doses of worms might rid people of inflammatory bowel disease, say researchers in Iowa. They believe this distressing condition, which is increasingly common in the developed world, is caused by the absence of intestinal parasites. "We're living in sterile boxes, breathing sterile air and drinking sterile water," says Joel Weinstock, who led the research.

Weinstock and his colleagues at the University of Iowa have already fed six sufferers eggs that hatched and developed into parasitic worms. The results were so dramatic that they are planning a larger trial this autumn. "Between the second and third week after treatment, five of the six patients went into complete remission," says Weinstock. A single dose of worms eased symptoms for about a month.

Inflammatory bowel disease—an umbrella term for ulcerative colitis and Crohn's disease—appears to be caused by an overactive immune system. Symptoms include diarrhoea, abdominal pain, bowel obstruction and bleeding.

Weinstock noticed that the rise in inflammatory bowel disease was preceded by a decline in intestinal worm infections. Seventy years ago, he says, 40 per cent of American children had worms such as *Ascaris lumbricoides*, which grow up to 20 centimetres long. As recently as the 1940s, many were infected with smaller whipworms (*Trichuris trichuria*). "By the 1960s, kids no longer had it," says Weinstock.

"The worms living in the gastrointestinal tract have been with us for 3 million years or longer," he says. "Our immune systems have grown used to their presence." And without such parasites, Weinstock believes the immune system is more likely to produce powerful inflammatory agents such as gamma-interferon, which fire up the activity of white blood cells called macrophages. "As we've dewormed, people have developed immune systems which are not damped," he says.

The six patients in the initial trial were chosen because steroids and other drugs designed to dampen down the immune system had not helped. Working with his colleagues David Elliott and Robert Summers, Weinstock gave each patient a drink containing microscopic eggs of a species of intestinal worm that doesn't normally infect people. Although these worms can survive in the human gut, growing to about 1 centimetre, they cannot reproduce and are eliminated after a couple of months.

Balfour Sartor, an expert on inflammatory bowel disease at the University of North Carolina in Chapel Hill, is intrigued by Weinstock's experiment. "It's an appealing way of using something that's of fairly low toxicity to treat a set of diseases that for now we don't have a cure for," he says. Sartor is himself experimenting with *Lactobacillus* and *Bifidobacterium* gut bacteria, again with the idea that they may have dampening effects on the immune system.

However, there is still a long way to go before doses of worms or bacteria emerge as an accepted treatment for inflammatory bowel disease. Larger controlled trials are needed to show that the results obtained so far aren't merely due to the placebo effect. **Andy Coghlan**

Gut reaction

- Ulcerative colitis affects up to 100 000 people in Britain, with 5600 new cases each year
- Up to 40 000 Britons are affected by Crohn's disease, and 3000 new cases are diagnosed each year
- Men and women are equally likely to suffer from inflammatory bowel disease

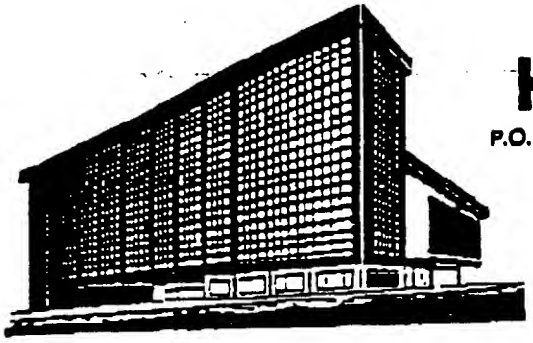
Source: National Association for Colitis and Crohn's Disease

Title: Science update: **Inflammatory bowel disease**

Summary: TV ADVERTS in the US ask the question "Got milk?" In future, they might add, "Got worms?" Researchers at the University of Iowa think that regular doses of worms might help cure **inflammatory bowel disease** by combating the absence of intestinal **parasites** in our "sterile" developed world. By the 1960s, most intestinal **parasites** were eliminated from children in the West - ending three million years of coexistence. "We're living in sterile boxes, breathing sterile air and drinking sterile water," Joel Weinstock, who led the research, told New Scientist. Weinstock and his colleagues fed six people with **inflammatory bowel disease** a drink containing microscopic eggs of a species of parasitic intestinal worm that does not normally affect people. Between 14 and 21 days later, five of the six went into complete remission, he said.

Source:	The Independent - London
Date:	08/06/1999
Price:	$2.95
Document Size:	Very Short (133 words)
Document ID:	PN19990809030021976
Citation Information:	FOREIGN; Features Section
Author(s):	Charles Arthur
Copyright Holder:	1999, Newspaper Publishing PLC
Document Type:	Article

319-353-6399



Houston Chronicle
P.O. Box 4260 Houston Texas 77210 (713) 220-7171

CITY DESK
FAX NO. (713) 220 6806

FAX TRANSMISSION COVER SHEET
DATE: Thurs Oct 7 TIME: ____

TO: Dr. Joel Weinstock c/o Claudia

ATTN: ____

DEPT: ____

FAX. NO. ____

FROM: Leigh Hopper, 713-220-6410

I am sending pages (including this page) 3

SPECIAL INSTRUCTIONS: Dr. Weinstock, would you mind fact-checking this? Also, can you please email us a photo of yourself (and even one of the worms if possible!) Thank you!

Leigh Hopper

leigh.hopper@chron.com

801 Texas Houston, Texas 77002

319-353-6399

{gutwormscopy} 10/07/99 16:12:07 [ctylah@edtsrv01]

By LEIGH HOPPER

Houston Chronicl Medical Writer

Am rican children 70 years ago had a closer relationship with dirt than they do now.

There were fewer sidewalks, paved roads and indoor toilets, and more kids spent more time playing outside in their bare feet. As a result of this daily contact with soil, almost all youngsters were infected with intestinal parasites such as hookworms, pinworms or whipworms.

And that was a good thing.

At least that's the thinking of Joel Weinstock, a University of Iowa researcher who believes regular doses of worms may be the key to treating people with inflammatory bowel disease, a serious and baffling disorder that affects at least a million people nationwide.

"As we move into our sterile boxes, (breathe) sterile air, we're no longer being exposed to some of the natural agents that may be required for optimal development of our immune systems," said Weinstock, director of the Digestive Disease Center at the University of Iowa Hospitals and Clinics. "As we've de-wormed, people have developed immune systems which are not damped."

The cause of inflammatory bowel disease, a term which encompasses both ulcerative colitis and Crohn's disease, is a mystery, but it is presumed to result from poor regulation of the intestinal immune system - an overreaction to normal intestinal bacteria.

The diseases usually begin in people during their late teens and 20s and last a lifetime. They cause abdominal pain, diarrhea and gastrointestinal bleeding. Sections of the intestine may become blocked by scar tissue and require surgical removal. Those afflicted are at greater risk for colon cancer.

In the United States and other industrialized countries, the disorder is rampant. However, the condition is rare in poor countries where parasitic worm infection is common.

Weinstock theorizes that man and lowly worm co-evolved to help each other. He b lieves intestinal worms, or helminths, dampen the immune response so they can thrive in humans. In the absence of these parasites, the human intestinal inflammatory response is unchecked and goes into overdrive.

Studies in mice performed supported the idea. Mice exposed to helminthic worms were protected from the development of inflammatory bowel disease.

This spring, working with colleagues David Elliot and Robert Summers, W instock gave six patient volunteers who hadn't responded to standard tr atments a drink laced with the microscopic eggs of a worm that doesn't normally infect people. Although these worms can live in the human intestine, r aching nearly a half-inch in length, they can't reproduce and are expelled after a couple of months.

Remarkably, after ing sting the eggs, all six patients improv d substantially. Five went into compl te remission. The treatment had no noticable side eff cts and the patients' improvements lasted about four weeks.

Dr. Atilla Ertan, director of the digestive disease department at Baylor College of Medicin , called the experiment "an unusual approach." He is

currently studying how a powder derived from the aloe plant may modulate the immune response in c litis. The study, at The Methodist Hospital, is free. To enroll, call 713-790-2171. [cq]

Dr. Alan Buchman, a professor of gastroenterology at University of Texa -Houston Health Science Center, called Weinstock's idea "an interesting hypothesis" but said a study of six patients is too small to say much. Treatments for Crohn's and colitis typically have a 30 to 35 percent placebo response rate, he said.

Weinstock's team is now organizing a clinical trial with additional patients to further investigate the treatment, compare it with a placebo and see if additional doses help.

"A year from now, you might look back and it means nothing," he said. "The bottom line is, it has potential. We will have to see before we can say for sure it's helping people. But we're encouraged."

SCIENCE NEWS
August 14, 1999
Vol. 156, No. 7
Pages 97-112

Germs of Endearment

Modern Hygiene's Dirty Tricks

The clean life may throw off a delicate balance in the immune system

By SIRI CARPENTER

Sweeping along 14th-century trade routes, an infectious agent left a trail of incomparable devastation throughout Asia and Europe. In China, this plague slashed the population from 125 million to 90 million by the century's end. In Cairo, the Black Death—so called because of the dark, swollen lymph nodes that characterize the disease—claimed 7,000 lives a day at its height. Before it subsided, the plague had wiped out one-third of Europe's population.

In most of the world today, the plague has receded to a distant, if gruesome, memory. So, too, at least in developed countries, have smallpox, typhoid fever, cholera, diphtheria, and polio declined. One by one, infectious diseases that once ravaged society and preyed especially on children have been quelled by better sanitation, antibiotics, and vaccinations.

While raising barricades against deadly scourges, however, the industrialized world has also shielded people from the microbes and parasites that do no harm. Does it matter?

A growing number of scientists now suspect that stamping out these innocuous organisms is weakening some parts of children's immune systems, allowing other parts to grow unchecked. Such an imbalance, they theorize, triggers a host of illnesses, including asthma, allergies, and even such autoimmune diseases as rheumatoid arthritis and the most severe type of diabetes.

This notion, called the hygiene hypothesis, arose from scientists' inability to explain the rising prevalence of asthma and allergies in many developed nations. The National Heart, Lung, and Blood Institute estimates that in the United States, for example, the incidence of asthma is now 1.75 times what it was in 1980, and for children less than 4 years old, 2.60 times the earlier incidence.

Pollution and allergens—such as mold and pollen—can take some of the blame, but not all of it. "One needs an explanation" for these trends, says Graham A.W. Rook of the University College London Medical School, who is one of the chief advocates of the hygiene hypothesis. "People should be getting healthier, not less healthy."

For several years, investigators have been uncovering signs that illness can result when the immune system lacks practice fighting bacteria and viruses. This evidence, however, has been circumstantial and too sparse to convince most scientists.

"It's greeted with some skepticism, and quite rightly, because we need more evidence," says Richard Beasley of the University of Otago's Wellington (New Zealand) School of Medicine. "In many respects, it's still early days, but the evidence is starting to build."

Recently, several epidemiological and experimental studies have converged to put the hygiene hypothesis on firmer ground. Some researchers are already trying to create vaccines that mimic potentially crucial immune effects of the microbes that society has banished.

Superstock

Too clean? Antiseptic surroundings may not allow a child's immune system to practice fighting off germs.

According to the hygiene hypothesis, the immune system is like a set of scales that sometimes tips sharply enough to send a person's health tumbling.

One arm of the immune system deploys specialized white blood cells, called Th1 lymphocytes, that direct an assault on infected cells throughout the body. Counterbalancing this, another arm of the immune system tries to hit the intruders even earlier. It produces antibodies that block dangerous microbes from invading the body's cells in the first place. This latter strategy exploits a different variety of white blood cells, called Th2 lymphocytes. The Th2 system also happens to drive allergic responses to foreign organisms.

At birth, an infant's immune system appears to rely primarily on the Th2 system. According to the hygiene hypothesis, the Th1 system can grow stronger only if it gets exercise, either through fighting infections or through encounters with certain harmless microbes. Without such stimulation—and ordinary colds and flu don't seem to do the trick—the Th2 system flourishes and the immune system teeters toward allergic responses.

Early support for this view came from Julian M. Hopkin, now at the University of Wales Swansea, and his colleagues. In 1997, they reported on a study of 867 Japanese children given a vaccine against tuberculosis. Those who showed a strong Th1 response—indicating previous exposure to the bacterium that causes the disease—had far fewer allergies and asthma than did those who didn't show a Th1 response.

Furthermore, among the children who had allergies, some showed a decrease in allergy symptoms after receiving the vaccine. The ones with a strong Th1 response to the tuberculosis vaccine were six to nine times as likely to benefit as were children who did not have such a response.

In the past, some scientists speculated that the Th1 system required periodic infections, particularly in childhood, in order to develop properly, but most researchers now dispute that idea. Rook

clean. He suspects that children need contact not with disease-causing agents but with innocuous microbes in sc d untreated water—particularly organisms called mycobacteria—to give the Th1 system enough of a workout.

"The [lymphocytes] have got to be kind of marinated in this stuff in the early years of life," he says. If they aren't, he says, the Th2 system grows ever stronger, priming the immune system to overreact to allergens.

Recent epidemiological research has further hinted that the cleanest environments may be the best breeding grounds for allergies and asthma. In the January JOURNAL OF CLINICAL AND EXPERIMENTAL ALLERGY, Swiss researchers reported that hay fever was less common for farm children than for urban children or for rural children who didn't live on farms.

Several years ago, scientists found that children in large families—particularly the younger siblings of brothers—had fewer allergies than children in small families did. Researchers speculated that exposure to the germs brought home by older siblings protected the younger children from allergies.

Bolstering that idea, a study in the Feb. 6 LANCET found that children from small families who entered day care before age 1 were less likely to develop allergies than those who entered day care later. No such difference emerged for children from larger families, suggesting that early day care may have stood in for the protection provided by dirty older siblings.

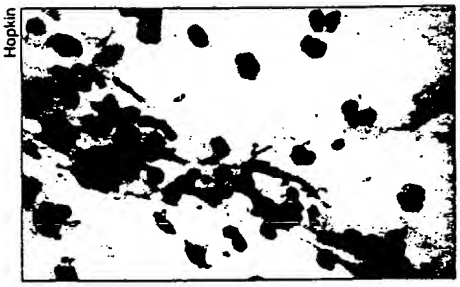
The antibiotics that thwart infectious diseases may also be spurring some immune disorders by killing off beneficial bacteria (SN: 11/22/97, p. 332). In the November 1998 THORAX, Hopkin and his colleague Sadaf Farooqi, now of Adenbrookes Hospital in Cambridge, England, reported that children who received oral antibiotics by age 2 were more susceptible to allergies than children who had no antibiotics, a finding that Beasley's group in New Zealand recently replicated.

The results, says Hopkin, may indicate that antibiotic treatment, which depletes the harmless bacteria within the gut, derails normal immune development in early life. A study in the May 1 LANCET by researchers in Sweden reinforced that idea: Children from families that avoid antibiotics and vaccinations have fewer allergies than other children do.

Encouraged by the epidemiological studies that support the hygiene hypothesis, some investigators are now trying to prevent illness by pumping up the Th1 system artificially. A team led by Stephen Holgate at the University of Southampton in England is conducting human trials of a Th1-inducing vaccine a preliminary analysis, the vaccine appears to dampen asthma patients' symptoms, the researchers announced last month. They should complete further immunological and clinical analyses by the end of September.

Despite promising advances, however, scientists acknowledge the limitations of the hygiene hypothesis. "We're desperately oversimplifying," says Rook. "We don't understand, really, why sometimes Th2 responses go crazy. Even I don't think [Th1-Th2 balance] is going to be the whole story. These are terribly complicated phenomena."

Hopkin



Mycobacteria (red) found in dirt and untreated water may help people cultivate a well-balanced immune system.

Without proper training early in life, some research suggests, the immune system can grow confused and lash out at inappropriate targets, including digested foods in the gut. At the University of Iowa in Iowa City, Joel V. Weinstock, David E. Elliott, and

contribute to the rising incidence of inflammatory bowel disease, a condition in which the lining of the intestines becomes chronically inflamed.

Unlike Rook, however, the Iowa researchers propose that the scales tip too sharply toward Th1 responses, leaving the Th2 response weakened. "Overall, I would disagree with Dr. Rook that we have severely altered our Th1 exposures," Elliott says. "It's true that we've limited our exposure to tuberculosis, and many of the viral agents have been controlled by vaccines. However, we still contact many, many viruses and bacteria that provide us with more than adequate Th1 experience."

Weinstock's group proposes that the Th1 dominance stems from a lack of parasitic worms called helminths. Despite parasites' bad reputation, the researchers contend that helminths are important members of the intestinal community. Throughout evolution, they say, the human immune system has grown to depend on helminths to suppress overly aggressive Th1 responses to bacteria, viruses, and dietary proteins. Because modern sanitation has largely eliminated intestinal parasites, the immune system sometimes begins to attack the lining of the gut.

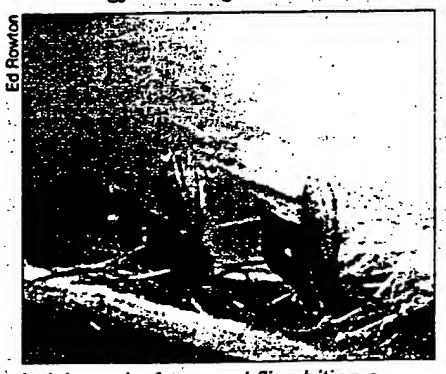
In May, the scientists reported at the annual meeting of the American Gastroenterological Association in Orlando, Fla., results of experiments in which they induced in mice a condition similar to inflammatory bowel disease. Mice deliberately infected with helminths, however, were protected from the disease. Collabo-

Fly bites help guard against *Leishmania*

The occasional bite of a blood-sucking fly may fine-tune the immune system and deter some infectious diseases.

Laboratory mice are best equipped to resist leishmaniasis—a tropical disease carried by sand flies—if they have had a little practice fending off disease-free flies, scientists reported in May at a meeting of the American Society for Microbiology in Chicago.

Ed Rowton



Leishmania-free sand flies biting a mouse ear may be arming the rodent against a later leishmaniasis infection.

David L. Sacks and Shaden Kamhawi of the National Institute of Allergy and Infectious Diseases in Bethesda, Md., twice exposed six laboratory mice to disease-free sand flies before introducing flies carrying *Leishmania* parasites. These exposed mice resisted infection better than did mice that had not been previously bitten by sand flies, the researchers found.

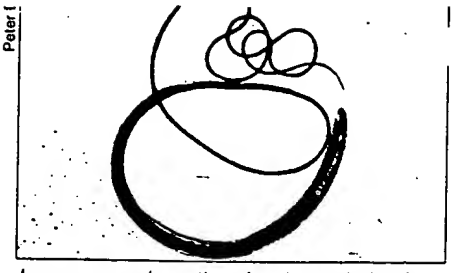
Sacks and Kamhawi propose that the saliva of flies that did not carry *Leishmania* may have stimulated the mouse immune systems, arming them against infection when they later encountered disease-carrying flies.

"It's fascinating work," says immunologist John R. David of the Harvard School of Public Health in Boston. "People who live in areas where they get leishmaniasis are obviously bitten a lot by sand flies, and this suggests that that in some ways protects them. People, however, still get the disease, but it might be much worse or affect more people if they had not been bitten by uninfected flies first." —S.C.

treatments for animals with autoimmune disorders, in which the immune system attacks parts of its own body.

The team has also begun treating a few patients suffering from inflammatory bowel disease by giving them a drink spiked with eggs from a harmless whipworm. Of six patients studied so far, all showed substantial improvement in their symptoms, the researchers reported at the May meeting.

The research is only an initial foray, the Iowa researchers caution, and controlled clinical trials are essential for evaluating the effectiveness of the treatment. Furthermore, they say, the precise role of Th1-Th2 balance in inflammatory bowel disease remains unresolved, as does the seeming contradiction between their research and the hygiene hypothesis' assumption that Th2 responses usually overpower Th1 responses.



Peter J. Bryant

Iowa researchers theorize that helminthic worms (adult female shown, approximately 60 millimeters long) keep people's immune systems from aggressively attacking the lining of their intestines.

By separating people from their dirty origins, the modern antiseptic environment may have also provoked the medical equivalent of friendly fire: autoimmune diseases such as rheumatoid arthritis and type I diabetes.

The radical notion that infrequent exposure to infectious agents contributes to autoimmune diseases has generated far more controversy than the idea that allergies and asthma stem from such deprivation. In fact, says Michael B. Oldstone of the Scripps Research Institute in La Jolla, Calif., most scientists hold the opposite view—that if anything, infections help drive autoimmune diseases (SN: 6/21/97, p. 380).

However, a group led by Irun R. Cohen at the Weizmann Institute of Science in Rehovot, Israel, believes it has evidence to the contrary. These researchers find that rats raised behind germ-free barriers germ-filled environments are.

According to Cohen, rats in the ultraclean environment don't develop the immune cells that can suppress autoimmune responses. If that's the case, he suggests, it may be possible to develop a vaccine to stimulate the aspects of the immune system needed to avoid autoimmune disorders.

"The immune system organizes itself through experience, just like the brain," Cohen argues. However, he notes, other factors, such as environmental toxins, probably also prompt autoimmune reactions. "I don't think cleanliness is the only problem. It's a complex system. The first thing is to ask the right questions, but we have to be patient about the answers."

Ultimately, it may be that asthma, allergies, and other immune disorders are the price society has to pay for escaping the appallingly virulent infectious diseases that have struck down children over the centuries. Scientists aren't quite ready to accept that proposition, however.

"We might be able to do something clever that can actually get the best of both worlds," says Beasley. "I think, at the end of the day, that will be the challenge, because we certainly don't want to go back to the days of old." □

Biology

From St. Louis, at the XVI International Botanical Congress

Oops. That mangrove tree's no lady

The supposedly female trees of the white mangrove have turned out to be perfectly good hermaphrodites.

This raises the question of why in the world there are male white mangroves, say Carol L. Landry of the University of Michigan in Ann Arbor and her colleagues. They estimate that less than 1 percent of flowering plants have both hermaphroditic and male plants, a mating system called androdioecy. In the past few years, botanists have reported this odd sex mix in a Japanese ash tree as well as in a member of the cucumber family.

The presumed female flowers of the white mangrove, *Laguncularia racemosa*, sport what look like male parts, but botanists had assumed that these organs didn't work. Landry tested that assumption in Florida by covering these flowers with small bags to seal out pollen from other trees. About half of the bagged flowers still managed to produce fruit.

Landry experimented with other crosses and found that the hermaphroditic flowers set more fruit when fertilized with pollen from all-male trees than when they self-pollinated.

In a field test on San Salvador in the Bahamas, Beverly J. Rathcke, also of Michigan, and Lee B. Kass of Elmira (N.Y.) College also found functional hermaphrodites, as well as males. —*S.M.*

Folk remedy zaps Ebola in lab test

A compound from the fruit of the bitter kola, a West African tree that healers have used for centuries to treat other diseases, stopped the Ebola virus from replicating in a laboratory test.

"The same forest that yields the dreaded Ebola virus could be the source of a cure," says Maurice Iwu, a descendant of a family of Nigerian healers who has trained in Western pharmacy. Iwu founded the Bioresources Development and Conservation Programme, with offices in Silver Spring, Md., which spearheads the investigation of compounds from the *Garcinia kola*. The National Institutes of Health has funded the identification of 46 potentially medicinal compounds from the tree. Some of these chemicals have quashed strains of flu virus in laboratory tests.

The Ebola virus, infamous as the fast-spreading epidemic in the movie *Outbreak*, first attracted the notice of Western doctors during a gruesome epidemic in Zaire in 1976. The virus kills by causing massive hemorrhaging from a wide range of organs. Neither Western nor African healers have a cure yet, and in some outbreaks 80 percent of the victims have died. —*S.M.*

How a bee finds its first buttercup

A bee that specializes in visiting buttercups relies on a just-for-newbies scent to help with its first attempts at flower identification.

European bees collected in the wild but raised in the laboratory away from real flowers get so excited by whiffs of a volatile compound from buttercups that they try to burrow through cheesecloth scented with the substance, reports Heidi E.M. Dobson of Whitman College in Walla Walla, Washington. Once the bees have some experience buzzing around buttercups, however, they no longer show a strong preference for the scent. "Seemingly, they've changed their search image," Dobson says. Learning a suite of other cues could make identification faster, she speculates.

Dobson has wondered for years how these specialized bees nail the right flowers. Their parents aren't around to provide taxonomy tips. Her earlier tests found that lab bees prefer the color yellow but come across many different yellow flowers.

Buttercup pollen releases bigger whiffs of the compound she tested than the rest of the flower does. The lab bees preferred buttercup pollen to offerings from other spring flowers.

Dobson has never tested the compound with cows, but she says it reportedly gives them taxonomy tips too, signaling a plant not worth munching. —*S.M.*

Parasitic worms ease abdominal pain in test

Early U of I work with Crohn's, colitis patients promising

By John Kenyon
Gazette Johnson County Bureau

IOWA CITY — Certain parasitic worms can help alleviate or possibly prevent the symptoms of Crohn's disease and ulcerative colitis, University of Iowa researchers announced Friday.

Both diseases affect sufferers in similar ways, causing abdominal pain, bleeding and diarrhea. People usually contract them in their late teens or early 20s, with the symptoms usually lasting their entire lives.

But subjects given helminthic worms saw their symptoms improve and in some cases disappear — a surprisingly successful outcome, said Dr. Joel Weinstock, a U of I professor and director of the project.

"We didn't expect to see a clinical response on the first try with one dose," Weinstock said.

The result, if proved in further studies, could lead to treatments or the development of drugs that could lessen or prevent the effects of the diseases, he said.

The study evolved from work Weinstock and others were doing to study the effect of parasitic worms on the immune system.

They found that certain diseases have increased in frequency since the 1930s, which coincides with steps taken to eliminate worms and other parasites from the body by creating more sterile environments.

In undeveloped countries, such diseases are rare, and 80 percent to 90 percent of children have parasitic worms in their systems, said Robert Summers, another researcher on the project.

But developed countries have virtually eliminated the worms, which have lived in humans for the past 3 million years, Weinstock said.

"We live in sterile boxes, eating sterile food and drinking sterile water," he said.

Dr. David Elliott, from the U of I, first tried reintroducing parasitic worms in mice. That trial found the worms seemed to give protection against dis ease.

That led to a human tria

■ Turn to 7A: W rm

Dr. Joel Weinstock
Director of U of I project

Cedar Rapids Gazette
Saturday, Aug. 7, 1999

Worms: Parasites ease colitis, Crohn's disease pain in U of I trial

■ From page 1A

where six patients with colitis or Crohn's disease were given a drink that contained microscopic worm eggs, each smaller than a grain of sand.

The eggs open in the gastrointestinal tract, and the larvae that are released attach themselves to the intestinal wall. The worms grow to about 1/16-inch long.

THE SYMPTOMS of all six subjects improved, and five of the six went into remission.

Eventually, that response ended, leading the researchers to postulate that the worms died and were passed from the system.

While the thought of intentionally ingesting worms may be less than appealing, Summers said the subjects were enthusiastic about the project.

"Of course this sounds crazy, outlandish, that you would suggest such a thing," he said. "And not everybody said yes."

One who did saw dramatic results.

Daniel McCabe, a graduate student at the U of I from New York, has had colitis for about nine years. When it would flare up, he would have to use the bathroom 10 to 12 times a day and he felt constant pain.

"It interfered with my life tremendously," he said.

AFTER THE treatment, he experienced "a night-and-day difference," he said, with his symptoms clearing after about two weeks. At times, he was in complete remission.

The cause of colitis is not known, but it appears to be a reaction of the immune system against the gastrointestinal system when the worms are not present, Weinstock said.

The researchers next will conduct a double-blind study in an attempt to prove their theory. They anticipate years of further study.

Weinstock said the long-term potential of the research could be a new way to treat not only colitis and Crohn's but other immune system-related diseases.

McCabe said the results were encouraging.

"There are treatment options out there that have very few side-effects and which can alleviate the symptoms like that," he said.

Thursday, September 23, 1999

War on Germs Is Enough to Make You Sick

■ Some researchers say the fight against infectious diseases is being waged at the expense of our immune systems. As exposure to such maladies drops, they say, susceptibility to things like bowel disease goes up.

By THOMAS H. MAUGH II, Times Medical Writer

The greatest medical achievement of the 20th century has been improved sanitation. Public health measures to block the spread of disease have saved more lives than all the drugs and medical procedures developed during the century combined.

A small but growing number of researchers are arguing, however, that in saving ourselves from cholera, dysentery, typhoid and a host of other infectious diseases, we may have set ourselves up for a sharp increase in autoimmune diseases.

Repeated exposure to a variety of germs early in life trains the immune system not to overrespond to immunostimulants encountered later in life, they argue.

In the absence of this training, the immune system becomes hyperreactive and mounts an attack against harmless chemicals or foods in the diet. In the process, they say, we become susceptible to such autoimmune diseases as Type 1 diabetes, rheumatoid arthritis, multiple sclerosis and asthma.

These diseases were virtually unknown before modern sanitary measures were introduced into the Western world and are still uncommon in developing countries. They are also more common in the colder, northern latitudes where infectious diseases spread less easily.

Now, another autoimmune disease is being added to the list: inflammatory bowel disease, which affects as many as 1 million Americans. Some researchers have suggested that changes in the bacterial population of the gut associated with improved sanitation are responsible for the dramatic increase in IBD in the latter half of this century.

Worms to the Rescue

But Dr. Joel Weinstock of the University of Iowa has a more radical idea. Experiments he has conducted on animals suggest that widespread exposure to intestinal worms in the early part of this century provided protection against development of IBD and that loss of this exposure through improved sanitary conditions has led to the onslaught of bowel disease.

Moreover, Weinstock and his colleagues have demonstrated in early experiments that infecting IBD patients with these worms--called helminths--can lead to remissions in patients with otherwise intractable bowel disorders. His team is now gearing up to perform much larger clinical trials of the technique.

"It is an intriguing concept that is still quite preliminary at the human disease level," said Dr. R. Balfour Sartor of the University of North Carolina, chairman of the Crohn's and Colitis Foundation of America. "But [Weinstock has] got reasonably good support in animal models . . . and it is certainly a promising area to investigate."

Researchers in Europe are already using unusual bacteria, such as lactobacilli and bisidobacteria, in experimental attempts to treat IBD by restoring the immune balance in the gastrointestinal system. Weinstock is simply using a different organism to achieve the same purpose.

"We are in the early stages of a whole new approach to treating bowel disease," said Dr. Dan Present of Mt. Sinai Hospital Medical Center in New York City. "If we can alter the environment [in the gut], perhaps we can diminish the immune response."

Inflammatory bowel disease is an umbrella term that encompasses two gastrointestinal problems--Crohn's disease and ulcerative colitis--that are in many ways two sides of the same coin. Crohn's normally occurs in both the small intestine and the large intestine, or colon, while ulcerative colitis is restricted to the colon. Ulcerative colitis affects only the innermost lining of the colon, while Crohn's affects all layers.

But the symptoms are very similar and include diarrhea, crampy abdominal pain and rectal bleeding. Each disorder is thought to affect about 500,000 Americans, both men and women equally, and they are usually diagnosed before the age of 30. About 20% to 25% of victims have a relative who is also affected, suggesting a genetic susceptibility to the disorders.

The primary treatments are aspirin-like drugs, steroids to suppress the immune system, and stronger immunosuppressive drugs, such as azathioprine and methotrexate. In more severe cases, surgeons remove the affected areas, but the problem usually returns in previously healthy segments of the gut.

Recent studies in several labs indicate that IBD results from an unbalanced immune response in the intestines involving two types of white blood cells, called T-helper-1 and 2, or Th1 and Th2. Th1 cells respond aggressively to foreign substances in the gut, while Th2 cells can dampen that response. In IBD, this system is thought to be out of balance, with the Th1 cells responding too aggressively to normal constituents of the intestines, damaging intestinal walls in the process. Weinstock's goal is to restore the normal balance.

He has worked with helminths for many years and discovered that they do something bacteria and viruses do not: They are potent stimulators of Th2 activity. He reasoned that people who were not infected by helminths during childhood did not experience this "Th2 conditioning" and were thus predisposed to IBD.

Testing the Theory

:periments in animals seemed to confirm this idea. Weinstock and Dr. David Elliott of Iowa used two groups of mice, one that was genetically engineered to develop colitis and one in which colitis could be induced with chemicals. Infecting both groups of animals with worms blocked development of the disease or, if applied after the disease had begun, induced remissions.

The researchers then obtained permission from the university's bioethics committee to use the worms to infect six people--four with Crohn's and two with ulcerative colitis. None of the patients were responding anymore to conventional treatments.

They used a strain of helminth that does not normally infect humans. Microscopic eggs were suspended in a glass of Gatorade that the patients drank. The half-inch-long worms colonized the gut for about four to five weeks before dying off and being excreted.

"Five of the six [subjects] went into clinical remission, and the one who didn't improved substantially," Weinstock said, and there were no side effects. The duration of the response was from one to five months, he added, and then the symptoms slowly returned. All of the patients "vigorously requested further dosing," he said.

Weinstock cautioned that this was a very preliminary study in a very small number of patients. "The patient wants it to work, so he may feel better even if there is no effect," he said. To overcome this "placebo effect" problem, he is now organizing a larger study, to be conducted at several major institutions across the country, in which the patients won't know if they received the worms or a placebo.

"We're trying to determine if it works objectively, scientifically, appropriately and without irrational exuberance," he said.

More information is available on the Crohn's and Colitis Foundation's Web site at http://www.ccfa.org and at the "virtual hospital" on the university's Web site, http://www.uiowa.edu.

Weekly Features

Iowa Researchers Unearth Potential Treatment for IBD: Intestinal Worms

Researchers at the University of Iowa College of Medicine are reporting some success in treating IBD with helminthic parasites, a type of intestinal worm. Joel Weinstock, M.D., Professor of Internal Medicine, David Elliott, M.D., Ph.D., Assistant Professor of Internal Medicine, and their colleagues are partly supported in their efforts by a CCFA Research Grant.

According to one theory on the origin of IBD, these diseases occur because of an overly aggressive immune response to normal intestinal contents. Two types of T cells interact in the intestine: T-helper-1 (Th1) cells, and T-helper-2 (Th2) cells. Normally, if Th1 cells respond aggressively, the Th2 cells can restore balance to the immune system in the intestine. Researchers believe that IBD is due to a Th1 response that gets out of hand.

Some helminthic parasites and their eggs live in the intestine; more than one-third of the world's population carries one or more of these organisms. They are potent stimulators of Th2 cells, helping to quiet Th1 activity. Dr. Weinstock and colleagues believe that failing to obtain helminths and experience this "Th2 conditioning" during childhood may predispose people to IBD.

This theory is supported by epidemiologic data, that is, who gets IBD. People are more susceptible to helminthic parasitic infections in rural, tropical, and lower-income societies, where people have more contact with the soil and conditions are less sanitary. IBD, however, is most prevalent in industrialized countries, where populations are at low risk for developing helminths.

In the first phase of their research, the University of Iowa researchers exposed mouse models to helminthic parasite eggs. Although the mice had been injected with trinitrobenzene sulfonic acid (TNBS), which normally causes colitis, they were protected from developing IBD. The group then developed a second animal model of IBD that also was protected by intestinal worms.

Following this success, the group moved on to a small study in humans. Four people with active Crohn's and two with ulcerative colitis, who had not responded to other treatments, received a single dose of helminthic eggs orally. The organism chosen was an animal helminth that can only colonize the human intestinal tract briefly. The eggs were produced in the laboratory and were shown to be free of disease-causing viruses or bacteria. All patients improved, experiencing a reduction in diarrhea and abdominal pain, and increases in energy levels. Five of six went into clinical remission. The improvements lasted from 1-5 months, suggesting that additional doses may be warranted. No adverse effects were seen.

Although the results of this small trial are encouraging, considerable study is required before helminthic parasites can be added to the list of treatments for IBD. The group is organizing a larger, double-blind trial (in which patients and physicians do not know whether they are taking the investigational drug or a placebo, an inactive substance).

The researchers have compiled a list of common questions and answers on the topic of heliminthic therapy. We will keep you posted as this research develops.

Date Posted: August 27, 1999

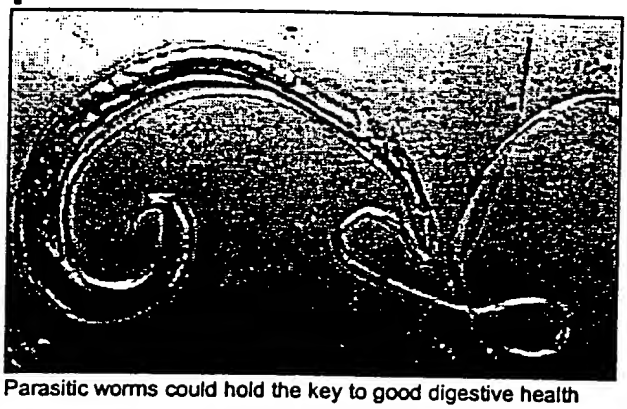
Front Page
World
UK
UK Politics
Business
Sci/Tech
Health
Education
Sport
Entertainment
Talking Point
In Depth
On Air
Archive

Feedback
Low Graphics
Help

Wednesday, August 4, 1999 Published at 17:52 GMT 18:52 UK

Health

Diet of worms solves gut problems



Parasitic worms could hold the key to good digestive health

Drinking live parasitic worms has been found to be an effective treatement for Crohn's Disease and Irritable Bowel Disease (IBD).

Researchers at the University of Iowa think that the virtual elimination of such creatures from the human gut over the years has left the body vulnerable to the massive immune reactions which typify the conditions.

Although only six sufferers took part in this trial, the results were so impressive that larger experiments could now follow.

All six were given a drink containing microscopic worms which can survive, although not reproduce in the human gut.

Between two or three weeks later, their symptoms completely disappeared, and stayed away for about a month.

A sterile existence

Dr Joel Weinstock, who carried out the tests, said: "We're living in boxes, breathing sterile air and drinking sterile water.

"As we've de-wormed, people have developed immune systems which are not damped.

"The worms living in the gastrointestinal tract have been with us for three million years, and our immune systems have got used to their presence."

Crohn's disease, ulcerative colitis and other forms of inflammatory bowel disease appear to be cause by an overactive immune system, which causes inflammation

Health Contents

- Medical not s
- Background Briefings

Relevant Stories

11 Aug 98 | Medical notes
Crohn's Disease: The facts

26 Nov 98 | Health
Asthma and bowel disease could have the same cause

04 Sep 98 | Health
Fast track development for Crohn's Disease Drug

17 Aug 98 | Health
Disfiguring disease blamed on fizzy drinks

Internet Links

IBD page

New Scientist

The BBC is not responsible for the content of external internet sites.

In this section

Germany urges calm in Ebola scare

Sun damage 'cuts cancer risk'

Balloon material in surgery breakthrough

'Trolleys cause back pain'

Health food shops 'give dangerous advice'

Nervous dental patients denied treatment

Schizophrenics 'denied new drugs'

Eating to succeed in sport

Understanding paranoia

Sexual athletes could wreck sporting car ers

Diet of worms solves gut problems

Dentists: Legalise teeth whitening

overactive ir ·une system, which causes inflammation in the diges. , system.

Symptoms include abdominal pain, bowel obstruction and bleeding.

The condition is incurable, and normal treatments include steroids, which can reduce the inflammation, although these have been known to produce side effects.

Dr Weinstock pointed out that the rise in such diseases over recent years has coincided with a reduction in the incidence of parasitic worms in humans.

Four tenths had worms

As little as 70 years ago, he said, 40% of US children enjoyed the company of worms which could grow up to 20 centimetres long.

Dr Mark Cottrill, a Lancashire GP with a special interest in IBD, said the use of worms was certainly novel.

He said: "I'm always open-minded about any innovation, even though treatments have become much better."

He said that he occasionally found harmless worms living in peace in his patients when he examined them with a colonoscope.

He said: "You do see these white things which don't like the light and wriggle away."

Other scientists, such as Dr Balfour Sartor, from the University of North Carolina, are experimenting with the use of bacteria to damp down the immune system in IBD patients.

He said of Dr Weinstock's work: "It's an appealing way of using something that's of fairly low toxicity to treat a set of diseases that for now we don't have a cure for."

Search
Advanced options | Search tips

Back to top | BBC News Home | BBC Homepage | ©

hitening
Sunscreen 'incr ases cancer risk'
Immune system 'worsens Alzheimer's'
Row over medical student 'crisis'
High lev l of trauma among Aborigines
Prostate cancer linked to mutant gene
Psychiatric disorders plague refugees
Fatal blood clot 'dismissed as panic'
Cot death rate falls
Smoking at any cost
Cancer study into artificial sweetener
CS gas used on psychiatric patients
Ebola cure hope
Coffee beats tea on heart disease
Pollution takes toll on healthy adults
Loud music threat to the young

London Times

August 5 1999 **BRITAIN**

Patients helped by dose of worms

BY NICK NUTTALL, TECHNOLOGY CORRESPONDENT

PEOPLE with inflammatory bowel diseases, including Crohn's disease, are being treated with doses of parasitic worms, with promising results.

Researchers claim that the worms naturally damp down the body's immune response so that it no longer attacks sensitive parts of a person's digestive system.

Inflammatory bowel diseases, such as Crohn's and ulcerative colitis, have become increasingly common in the Western world.

At the same time, the level of parasitic worm infections has dwindled to virtually nothing, with most people in industrialised countries now "worm free".

Joel Weinstock, who has led the research, told *New Scientist* magazine: "We're living in sterile boxes, breathing sterile air and drinking sterile water."

His group, based at the University of Iowa, has been testing a theory that, in some people, parasitic worms are vital for controlling the body's immune response. It is likely that the worms produce proteins or compounds that block the production of gamma-interferon or some other powerful, inflammatory agent.

"The worms living in the gastrointestinal tract have been with us for three million years or longer. Our immune systems have grown used to their presence," Dr Weinstock said.

Seventy years ago, 40 per cent of children in the West had worms such as Ascaris lumbricoides or whip worms, Trichuris trichuria. However, by the 1960s, children in the West no longer suffered from worms, save for the occasional infestation of tiny pin worms, which can make bottoms itchy.

The researchers fed six sufferers eggs of parasitic worms, which hatched out. "Between the second and

Next page: Ultrasound - for your ears only

third week after treatment, five of the six patients went into complete remission," Dr Weinstock said.

The patients were chosen because steroids and other drugs designed to damp down the immune system had failed to work.

The type of worm chosen does not normally infect people and cannot reproduce in the human digestive system, so, after growing to around one centimetre long, the worms died and were excreted. The scientists are planning larger trials to test the idea.

Yesterday Paul Garside, an immunologist at the University of Glasgow, said that there were risks attached to feeding people parasitic worms. The parasites can carry their own diseases, which can be harmful to man.

However, Dr Garside said that the work might lead to new ways of treating sufferers. He said that it might be possible to isolate those substances produced by the worms that moderate the immune response and to turn them into drugs.

Next page: Ultrasound - for your ears only

Arts (Mon - Fri) | Books (Sat) (Thu) | British News | Business | Court page | Features (Mon - Fri) | *Go* (Sat) | Metro (Sat) | Obituaries | Interface (Wed) | Opinion | Sport | Travel (Sat) (Thu) | Vision (Sat) | Weather | Weekend (Sat) | Weekend Money (Sat) | World News

The Boston Globe boston.com

Nation | World

[Send this story to a friend | Easy-print version | Add to Daily User]

A little intestinal fortitude

By Reuters, 08/05/99

LONDON - Regular doses of microscopic worms might treat inflammatory bowel disease, by combating the absence of intestinal parasites, New Scientist magazine said yesterday.

"We're living in sterile boxes, breathing sterile air and drinking sterile water," Joel Weinstock of the University of Iowa was quoted as saying in New Scientist.

Weinstock and his colleagues fed six people with inflammatory bowel disease a drink containing microscopic eggs of a species of intestinal worm that does not normally affect people.

The results were so dramatic that he is planning a larger trial this year.

"Between the second and third week after treatment, five of the six patients went into complete remission," Weinstock said.

Inflammatory bowel disease is an umbrella term for incurable bowel illness such as Crohn's disease and ulcerative colitis. Patients suffer from diarrhea, fever, abdominal pain, and weight loss. With drugs to control the condition, they can lead normal lives.

Weinstock had noticed that the rise in inflammatory bowel disease was preceded by a decline in intestinal worm infections.

Seventy years ago, 40 percent of American children had worm infections, but by the 1960s, they no longer did, he said in New Scientist.

"The worms living in the gastrointestinal tract have been with us for 3 million years or longer. Our immune systems have grown used to their presence," he said.

Without such parasites, Weinstock said, the immune system was more likely to produce powerful inflammatory agents. Bowel disorders appear to be caused by an overactive immune system.

The worms Weinstock fed his patients grow to around 1 centimeter long, but they cannot reproduce and are eliminated after a couple of months.

New Scientist, citing the need for larger controlled trials, said there was still a long way to go before worms would be accepted as treatment for

RELATED COVERAGE
A week of stories from the Boston Globe's Washington bureau

LATEST NEWS
National
International
Washington, D.C.

Sections
PAGE ONE
NATION | WORLD
METRO | REGION
BUSINESS
SPORTS
LIVING | ARTS
EDITORIALS | OP-ED

Weekly
Health | Science (Mon.)
Food (Wed.)
Calendar (Thu.)
At Home (Thu.)
Picture This (Fri.)

Sunday
Automotive
Cape & Islands
Focus
Learning
Magazine
New England
Real Estate
Travel
City Weekly
South Weekly
West Weekly
North Weekly
NorthWest Weekly
NH Weekly

Features
Archives
Book Reviews
Columns
Comics
Crossword
Horoscopes
Death Notices
Lottery
Movie Reviews

still a long way to go before worms would be accepted as treatment for inflammatory bowel disease.

This story ran on page A22 of the Boston Globe on 08/05/99.

[Send this story to a friend | Easy-print version | Add to Daily User]

Thursday August 5 1:17 AM ET

Hard To Swallow? Worms Could Cure Bowel Disorders

LONDON (Reuters) - Regular doses of worms might help cure people of inflammatory bowel disease by combating the absence of intestinal parasites in our sterile world, New Scientist magazine said Wednesday.

``We're living in sterile boxes, breathing sterile air and drinking sterile water,'' Joel Weinstock of the University of Iowa told New Scientist.

Weinstock and his colleagues fed six inflammatory bowel disease sufferers a drink containing microscopic eggs of a species of intestinal worm that does not normally affect people.

The results were so dramatic that he is planning a larger trial later this year.

``Between the second and third week after treatment, five of the six patients went into complete remission,'' Weinstock said.

Inflammatory bowel disease is an umbrella term for incurable bowel illness Crohn's disease and ulcerative colitis. Patients suffer from diarrhea, fever, abdominal pain and weight loss. With drugs to control the condition, they can lead normal lives.

Weinstock came up with the unorthodox treatment when he noticed that the rise in inflammatory bowel disease was preceded by a decline in intestinal worm infections.

Seventy years ago, 40 percent of American children had worm infections but by the 1960s, they no longer did, he told New Scientist.

``The worms living in the gastrointestinal tract have been with us for three million years or longer. Our immune systems have grown used to their presence,'' he said.

Without such parasites, Weinstock said the immune system was more likely to produce powerful inflammatory agents. Bowel disorders appear to be caused by an overactive immune system.

The worms Weinstock fed his patients survive in the human gut, growing to lengths of around one centimeter, but cannot reproduce and are eliminated after a couple of months.

New Scientist said there was still a long way to go before worms were accepted as treatment for inflammatory bowel disease as larger controlled trials were needed.

Weinstock, Joel

From: Moore, Tom
Sent: Thursday, October 28, 1999 2:41 PM
To: Weinstock, Joel
Subject: RE:

Dr. Weinstock,
Locally, the story was carried by KCRG-TV, KGAN-TV, KWWL-TV, WMT-AM & FM, Des Moines Register, Cedar Rapids Gazette, Daily Iowan, Iowa City Press-Citizen, WHO-TV, KCCI-TV, WOI-TV, WQAD-TV, KWQC-TV, WHBF-TV, KIMT-TV, and KTVO-TV. I will get to you copies of all of the national and international coverage that I am aware of, including the Discovery Channel in Canada, SAT-1 TV in Germany, and National Geographic - Explorer's Journal in the UK. Tom M.

----Original Message----
From: Weinstock, Joel
Sent: Thursday, October 28, 1999 2:26 PM
T : Moore, Tom
Cc: 'Kathy Williams'; 'Daniel Happe'; Elliott, David
Subject: RE:

Please send me a copy of whatever you have. Thanks

I guess we need to know from the attorney if we need to spend the money to obtain a comprehensive list.

Joel

----Original Message----
From: Moore, Tom
Sent: Thursday, October 28, 1999 1:49 PM
To: Weinstock, Joel
Subject: RE:

Dr. Weinstock,
I have been saving clips as we've gone along. Our consulting firm in Chicago did provide a partial list as well. I can provide everything I have. Should I send the list to you or directly to the patent attorney and copy you?
Compiling a comprehensive list would be challenging, to say the least. Even if we engaged the services of a national clipping service for several thousand dollars, we would not know the full extent of the coverage as those services typically find only about one-half of all the stories in question. Searching the internet would be another possiblity, but it would be time-consuming. Just let me know what you would like me to do next. Thanks, Tom M.

----Original Message----
From: Weinstock, Joel
Sent: Thursday, October 28, 1999 1:08 PM
To: Moore, Tom
Cc: Elliott, David; Bishop, Claudia
Subject:

Dear Tom,

The University patent lawyer for the parasite-IBD project wishes a complete list of publications (newspapers, magazines, radio and TV) who carried the story and the dates. I realizes that this now may be in the hundreds. Is this obtainable via computer search? Many papers carried the Reuters and Associated press story. Newspapers are even running the story now. For instance, a story on this appeared in Cincinnati about 2 weeks ago. There were stories in Poland, Croatia, South Africa, etc. I found out because of the incoming e-mails. Any suggestions on how we can get a handle on this?

Joel

PW 379-0705 Wormy 9/9/99 11:44 AM Page 1

Clinical Update

Job No: PW 379-0705
Job Title: Wormy
Stage: 3
Initials: lm

WORMY Rx

Parasites subdue autoimmune disease

ORLANDO—When the worms crawl in, Crohn's, et al. may bow out.

Observing that such autoimmune diseases are rare in developing nations where parasitic worms are prevalent, a University of Iowa physician reasoned that a common helminth might help modulate the immune system.

Reporting on a pilot study, Dr. Joel Weinstock said, four patients with refractory Crohn's and two with ulcerative colitis had remissions lasting months after drinking reagent-grade porcine whipworm eggs. The parasite can't reproduce in the human gut, he told the American Gastroenterological Association meeting here.

The worms may have triggered a response that promotes Th2 cells and suppresses Th1 cells, he suggests. —*Judith Groch*

Moore, Tom

Fr m: Weinstock, Joel
Sent: Wednesday, September 01, 1999 4:18 PM
T : Moore, Tom
Subject: RE: SAT1-TV Itinerary

Thanks for your help!!

Joel

----Original Message----
Fr m: Moore, Tom
Sent: Wednesday, September 01, 1999 4:14 PM
T : Elliott, David; Summers, Robert; Weinstock, Joel
Cc: Borg, Eldean; Buchheim, Tom; Hollensbe, Jim; Homan, Eve; Kupka, Chuck; Liddell, Rita F.; Lundell, Diana K.; Maddy, Coleen; Sondergard, Michael L.; Yank, Ted; Vice, Sharon
Subject: SAT1-TV Itinerary

I just spoke with Gary Wahlgren from the SAT1-TV (Germany) to confirm his plans. Gary is arriving late tonight. He will be staying at the City Plaza Hotel (formerly to downtown Holiday Inn). He will meet his crew here at the hospital's main entrance at 10:00 AM tomorrow, Thursday, Sept. 2, 1999. The tentative schedule from that point is as follows:
10:00 AM - Scout interview locations.
10:30 AM - Set up for Dr. Weinstock interview.
11:00 AM - Record Dr. Weinstock interview.
11:30 AM - Record Dr. Weinstock B-roll, including simulated helminthic therapy treatment.
12:30 PM - Lunch break for crew.
1:30 PM - Accompany Dr. Elliott to Electron Microscopy Facility for B-roll.
3:00 PM - Obtain supporting video, i.e. DDC, exteriors, lab scenes, etc.
5:00 PM - Wrap and depart.

I am providing the crew dubs of the Daniel McCabe interview with Dr. Summers, the video we shot with Dr. Elliott, and the colonoscopic video of intestinal parasites, in addition to the background material previously prepared.

I will escort the crew and assist with the production coordination. Please let me know if any of you have any questions or concerns. Thank you, Tom Moore JOPMC 6-3945

UNIVERSITY of IOWA HEALTH CARE

University of Iowa Health Care

Joint Office for Planning, Marketir
and Communications
8798 JPP
200 Hawkins Drive
Iowa City, Iowa 52242-1009
319-356-1009 Tel
319-356-2106 Fax

September 20, 1999

To Whom It May Concern:

I am Tom Moore in the Joint Office for Planning, Marketing and Communications, University of Iowa Hospitals and Clinics, Iowa City, Iowa. I am sending two videotape cassettes (Beta SP format) to Martin Leigh, Researcher, Explorer's Journal, National Geographic TV, Mentorn Barraclough Carey, 140 Wardour Street, London W1V 4LJ, UK for the purpose of producing a broadcast television program. The items are valued at $500, and are not for resale.

Tom Moore

Tom Moore
Joint Office for Planning, Marketing and Communications
University of Iowa Hospitals and Clinics
200 Hawkins Drive Room 8798 JPP
Iowa City, IA 52242-1009
USA

Moore, Tom

From: Lois Riggan [lois-riggan@uiowa.edu]
Sent: Tuesday, August 31, 1999 3:14 PM
To: National News Highlights Recipient
Subject: August 1999 National News Highlights

AUGUST 1999

NATIONAL NEWS HIGHLIGHTS

A Monthly Summary of UI News in the National Media

Produced by University News Services

Please note: Internet access to the full text of articles summarized below may require on-line subscriptions to the publication in some instances.

DANCE MAGAZINE, September 1999 -- **HANCHER AUDITORIUM** teamed up with public libraries in Iowa City, Cedar Rapids and Lisbon and area churches to conduct an audience development residency with the Colorado String Quartet to increase audiences for chamber music among churchgoers and library-users. About 1,200 people took part in listening and discussion programs.

DANCE MAGAZINE, September 1999 -- **JUDITH HURTIG**, assistant director of Hancher Auditorium, contributed an article to the magazine on the University of Iowa's involvement with the Colorado Quartet for seven weeks in the fall and winter of 1997. Hancher teamed with other local groups to promote interest in string quartet music.

DANCE MAGAZINE, September 1999 -- The University of Iowa's Hancher Auditorium is spending nearly $1 million in preparation for its Millennium Festival, which will feature 15 major commissions. Eight are in dance, with the remaining seven divided between theater and music. "I think the international dance community will be bowled over by that kind of money being spent on dance commissions," said **WALLACE CHAPPELL**, director of Hancher. Hancher's upcoming season will feature new works by a variety of artists, including UI alumnus **LAR LUBOVITCH**. Lubovitch will pair up with the American Ballet Theatre for performances Nov. 2 and 3.

AMERICAN THEATRE, September 1999 -- "Geometry of Miracles," Quebecois director Robert Lepage's latest creation, will make its American premiere Sept. 9-11 at the **HANCHER AUDITORIUM** in Iowa City before visiting Minneapolis, Columbus, Ohio, and New York. The play dramatizes the later years in the life of the great American architect Frank Lloyd Wright.

NEW YORK TIMES, Aug. 31 -- **JOEL WEINSTOCK**, M.D., of the University of Iowa College of Medicine, is identified as the lead researcher in a study in which the eggs of parasitic worms were ingested by patients to treat acute, chronic inflammatory bowel disease. "Every one of those patients is begging to be re-treated," said Weinstock. The preliminary results

NEW YORK TIMES, Aug. 17 - On the morning of the Iowa Straw Poll, Elizabeth Dole flew to Iowa City to greet 300 fraternity and sorority members from the **UNIVERSITY OF IOWA** before they boarded buses to Ames, where the poll was held. The bus caravan, paid for by the Dole campaign, was organized by the UI Tri-Delta chapter.

SLATE, Aug. 17 -- As part of its ongoing coverage of the 2000 presidential election, Slate will run daily results from the University of Iowa's **IOWA ELECTRONIC MARKET** alongside its own Pundits Index. The IEM trades "stock" in candidates. As Slate writes: "It's slightly complicated, but the point is to see if the invisible hand of capitalism can beat the gasbags at predicting election results."

http://www.slate.com:80/Readme/99-08-17/Readme.asp

LOS ANGELES TIMES, Aug. 16 -- Stephen Brogden, who has a bachelor's degree

in English and master's degree in library science from the **UNIVERSITY OF IOWA**, is profiled about his recent appointment as interim director of the Thousand Oaks Library in California.

PHILADELPHIA INQUIRER, Aug. 16 - The outcome of the Iowa Straw Poll reflects a divide within the Republican Party. Roughly half of the votes went to establishment candidates with the other half going to party conservatives. **ARTHUR MILLER**, a UI political science professor, said late Saturday night: "It's a divide between the pragmatists and the moralists, and it will keep appearing down the road. No matter how much money and resources Bush has, he is going to have to confront it at some point, because, long-term, his relations with the [movement conservatives] will be a major factor in Republican politics next year."

http://www.phillynews.com/inquirer/99/Aug/16/front_page/IOWA16.htm

LOS ANGELES TIMES, Aug. 16 - A column about recent innovations in health care mentions the **UNIVERSITY OF IOWA** study that found that patients who suffer from inflammatory bowel diseases can get relief by ingesting a certain type of parasitic worm.

SAN DIEGO UNION-TRIBUNE, Aug. 15 - The Iowa Straw Poll results will likely lead some candidates to rethink their bid for the Republican presidential nomination. "The straw poll results will end up sucking the blood out of a lot of campaigns," said **ARTHUR MILLER**, a UI professor of political science.

CHRISTIAN SCIENCE MONITOR, Aug. 15 - Although Texas Gov. George Bush won Saturday's Republican Straw Poll in Ames, some say that considering the enormous amount of resources his campaign dedicated to the run up to the poll, Bush didn't do as well as he should have in winning only 31 percent of the vote. "He didn't even get close to 50 percent," says **ARTHUR MILLER**, a UI political science professor.

http://www.csmonitor.com/durable/1999/08/16/fp3s1-csm.shtml

INFORMATION TODAY, Aug. 15 -- **BARBARA I. DEWEY**, director of information and

http://www.amcity.com:80/albany/stories/1999/08/09/story7.html

CBS.COM, Aug. 9 -- Regular doses of worms might help cure people of inflammatory bowel disease by combating the absence of intestinal parasites in our sterile world, according to the results of a study led by University of Iowa professor of internal medicine **JOEL WEINSTOCK** and published in a recent issue of New Scientist magazine. Researchers fed six inflammatory bowel disease sufferers a drink containing microscopic eggs of a species of intestinal worm that does not normally affect people. The results were so dramatic that he is planning a larger trial later this year.

SCIENCE DAILY, Aug. 9 - A team of **UNIVERSITY OF IOWA** engineers is studying how conventional airbags work in order to help researchers design safer airbags for new cars and trucks. P. Barry Butler and L.D. Chen, professors of mechanical engineering and project co-principal investigators, are in the final year of a three-year, $369,000 General Motors grant funded through the National Highway Traffic Safety Administration. Chen says that the primary goal of the UI project is to understand the physics of auto airbags.

http://www.sciencedaily.com:80/releases/1999/08/990809081306.htm

THE IRISH TIMES, Aug. 9 -- Regular doses of worms might help cure people of inflammatory bowel disease by combating the absence of intestinal parasites in our sterile world, **JOEL WEINSTOCK** of the University of Iowa told New Scientist magazine. Weinstock and his colleagues fed six inflammatory bowel disease sufferers a drink containing microscopic eggs of a species of intestinal worm that does not normally affect people. The results were so dramatic that he is planning a larger trial later this year. Various versions of this story also were reported Aug. 5 by **UPI** in its "Domestic

News/Health Tips"; the **CALGARY HERALD**; **THE HERALD** (Glasgow); **THE JOURNAL** (Newcastle); **THE OTTAWA CITIZEN**; **THE VANCOUVER SUN** and **WESTERN DAILY PRESS**; on Aug. 6 by **THE INDEPENDENT** (London); on Aug. 7 by the **ASSOCIATED PRESS** state and local wire service and in **NEW SCIENTIST**. The story also received broadcast coverage, including Aug. 4 by **KCOP-TV** (UPN) in Los Angeles; **WLTV-TV** (Univision) in Miami; on Aug. 6 by **KMBC-TV** (ABC) in Kansas City; **WSYX-TV** (ABC) in Columbus and **KTNV-TV** (ABC) in Las Vegas; on Aug. 8 by the **ALL NEWS CHANNEL**; Aug. 9 by **WLS-TV** (ABC) in Chicago; **KTRK-TV** (ABC) in Houston; **KOMO-TV** (ABC) in Seattle/Tacoma; **WRTV-TV** (ABC) in Indianapolis; **WTNH-TV** (ABC) in Hartford/New Haven; **WGGB-TV** (ABC) in Springfield/Holyoke; **KOCO-TV** (ABC) in Oklahoma City; **WPBF-TV** (ABC) in West Palm Beach/Fort Myers; **WJRT-TV** (ABC) in Flint/Saginaw; **KNXV-TV** (ABC) in Phoenix; **WGNO-TV** (ABC) in New Orleans; **WOKR-TV** (ABC) in Rochester, N.Y.; **WROC-TV** (CBS) in Rochester, N.Y; and on Aug. 10 by **WRTV-TV** (ABC) in Indianapolis; **WSOC-TV** (ABC) in Charlotte; **KNXV-TV** (ABC) in Phoenix and **WTAE-TV** (ABC) in Pittsburgh.

HEALTHSCOUT, Aug. 9 -- Scientists with the **UNIVERSITY OF IOWA** believe they understand part of what causes progeria, a disease that ages children rapidly, often killing them before they reach 14. The UI researchers found that people with progeria are born with a deficiency in two enzymes that protect cells against damage from oxidative chemicals, also known as free radicals. The team eventually plans to use a genetically engineered virus to deliver genes for the two enzymes into progeria patients to produce more of the protective enzymes.

CHRONICLE OF HIGHER EDUCATION, Aug. 5 -- Mentioned in a daily report sent via email to subscribers of the Chronicle is the fact that poet **JORIE GRAHAM** of the University of Iowa Writers' Workshop is listed among Talk magazine's "50 big mouths we hope will never shut up." The premiere issue of the magazine also lists among the top 50 rappers Chuck D, the Rev. Jesse Jackson, poet Maya Angelou and Catharine MacKinnon, the University of Michigan Law School's lawyer and feminist scholar. The article is not available on-line, but information about the magazine may be found on its World-Wide Web site at http://www.talkmagazine.com

REUTERS, Aug. 5 -- Regular doses of worms might help cure people of inflammatory bowel disease by combating the absence of intestinal parasites in our sterile world, **JOEL WEINSTOCK** of the University of Iowa told New Scientist magazine Wednesday. Weinstock and his colleagues fed six inflammatory bowel disease sufferers a drink containing microscopic eggs of a species of intestinal worm that does not normally affect people. The results were so dramatic that he is planning a larger trial later this year.

http://dailynews.yahoo.com/headlines/sc/story.html?s=v/nm/19990805/sc/health_worms_2.htm

The same wire story appeared Aug. 5 on the **BOSTON GLOBE** web site at:

http://www.boston.com/dailyglobe2/217/nation/A_little_intestinal_fortitude+.shtml

Another version of the story appeared Aug. 5 on **THE TIMES OF LONDON** web site at:

http://www.the-times.co.uk/news/pages/tim/99/08/05/timnwsnws01020.html?999

Another version of the story appeared Aug. 4 on the **BBC NEWS** web site at:

http://news.bbc.co.uk/hi/english/health/newsid_412000/412142.stm

The Reuters version of the story appeared Aug. 4 on the **FOX NEWS** web site.

USA TODAY, Aug. 5 -- Some residents who live near a **UNIVERSITY OF IOWA** incinerator that is used to burn animal carcasses after medical research fear it gives off radiation and want it shut down. Many of the carcasses, which were used for cardiovascular research, were exposed to radiation. Officials said the incinerator is safe.

http://www.usatoday.com/news/states/iamain.htm

THE TIMES OF LONDON, Aug. 5 -- Richard Olney, who attended the **UNIVERSITY OF IOWA** at his father's insistence and was one of the first food writers to introduce the simple joys of French country cooking to American readers; was found dead Tuesday at his home at Sollies-Toucas in Provence. He was 71.

http://www.the-times.co.uk/news/pages/tim/99/08/05/timobiobi02003.html?999

Weinstock, Joel

S nt: Monday, November 01, 1999 8:57 AM
To: Weinstock, Joel
Subject: FW: Treatment for bowels disease

—Original Message—
From:
Sent: Monday, November 01, 1999 8:56 AM
To: '=?UNKNOWN?Q?Andr=E9_=C9mond?='
Subject: RE: Treatment for bowels disease

Dear Andre,
I am very sorry to hear about your difficult problem and also distressed that we have such limited resources to conduct our research. We are looking for funding for the research, but at this time, our center is the only center that is conducting studies on this agent. We are embarking on a larger trial at The University of Iowa which will be a proper scientific study to establish whether the therapy is useful or not. If it is, we will be looking to establish larger studies and it is possible that Canadian centers will be included. In the meantime, all that I can suggest is to be certain that every avenue of treatment has been explored. There are excellent centers in Toronto and Hamilton which could be helpful in managing your disease.

—Original Message—
From: =?
S nt:
To:
Subject:

Dear Mr. Summers,

I saw a television interview that you gave on the Discovery Channel. It was about your new treatment for bowels disease.

To go directly to the point, is it possible for a Canadian to try this treatment? I don't think I have time to wait the three or four years required to commercialise your discovery.

I'm a little bit desperate; at 43, I have been through two surgery and was left with half of my colon. Drugs don't appear to work anymore. My last prednisone treatment caused the beginning of a cataract (as I teach for a profession, it was worrysome, to say the least). Antibiotics work marginally. I have to receive iron injections to compensate the lost of blood.

Whatever your answer, I want you to know that I appreciate very much your work. It gives hope.

EXHIBIT "C"

Cytokines that regulat autoimmune responses

Marika Falcone and Nora Sarvetnick*

Autoimmune responses are controlled by complex regulatory circuits. Previous work has revealed that factors controlling autoimmunity can act both as potentiating and inhibitory agents, depending upon the site and timing of exposure. Recent advances in this complex field have at least partially uncovered the mechanism whereby these regulatory molecules participate in autoimmune processes. IL-12 production in the absence of infection may predispose to autoimmunity. IL-4 and transforming growth factor β may suppress autoreactive T cells. Proinflammatory cytokines may ameliorate autoimmunity, dependent on the timing and level of production. In many cases, cytokines may act via antigen-presenting cells.

Addresses
Department of Immunology, IMM-23, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA
*e-mail: noras@scripps.edu

Current Opinion in Immunology 1999, 11:670–676

Abbreviations

APC	antigen-presenting cell
DC	dendritic cell
EAE	experimental allergic encephalomyelitis
GM-CSF	granulocyte/macrophage-colony-stimulating factor
IDDM	insulin-dependent diabetes mellitus
IFN-γ	interferon γ
IL-12R	IL-12 receptor
ins	human insulin promotor
MBP	myelin basic protein
RIP	rat insulin promoter
TGF-β	transforming growth factor β
TNF	tumor necrosis factor

Introduction

The role of cytokines in the pathogenesis of T-cell-mediated diseases has been the object of intense investigation in the past few years. Transgenic strains have been generated, each expressing individual cytokines in organs targeted by autoimmune diseases. These animal models provide the most advanced tool available for analyzing the relationship between cytokines and T-cell-mediated autoimmune responses. In particular, such experiments suggest that the local expression of cytokines regulates autoimmune responses by modulating the activation of distinct cell populations. In this review, we will analyze mechanisms underlying the regulation of autoimmunity by cytokines through their effects on cellular subsets involved in the pathogenesis of T-cell-mediated autoimmune diseases.

Cytokines dir ctly regulate s lf-reactive T cells to balance effective immune resp nses with the avoidance of autoimmunity

The classical Th1/Th2 paradigm in autoimmunity

An autoreactive T cell repertoire is part of a healthy immune system and it could be frequently activated in the course of common infections [1]. Cytokines seem to have a key role in this process by providing the necessary signals to turn on/off T cells specific for self antigens. A widely held belief is that, when the cytokine profile of autoreactive T cells shifts toward an inflammatory Th1 type, the result is pathogenicity and autoimmune diseases [2,3]. IL-12, a critical cytokine for induction of Th1 immune responses [4–6], was found to be essential for T-cell-mediated autoimmunity [7,8]. Specifically, IL-12 administration worsened autoimmune phenomena by inducing the differentiation of Th1 autoreactive cells [9,10] whereas the lack of IL-12 in genetically deficient mice or mice treated with anti-IL-12 antibody ameliorated experimental models of autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM) in NOD mice [11,12], experimental allergic encephalomyelitis (EAE) [13,14], experimental autoimmune uveitis [15,16] and collagen-induced arthritis [17].

IL-12, secreted by antigen-presenting cells (APCs) soon after infection, could represent a signal that the host requires a strong inflammatory immune response [18] — thus inducing the activation and Th1 differentiation of autoreactive T cells [19]. However, autoreactive T cell responses generated by IL-12 in the absence of danger might predispose to self-destructive immunity [20]. Support for this theory is the new-found link between genetic susceptibility to autoimmune diseases and the ability of self-reactive T cells to directly induce IL-12 secretion by APCs, thus bypassing any need for infectious agents [21••]. Evidently the self-induced expression of sufficient levels of the β2 subunit of the IL-12 receptor (IL-12R) to provoke Th1 differentiation by autoreactive T cells in autoimmune-prone mice, by itself, produces unnecessary, even destructive, inflammatory responses.

On the other hand, there are several cytokines belonging to the Th2 pathway that directly downmodulate Th1 autoimmune responses and, thereby, dampen T-cell-mediated autoimmunity. In fact, both IL-4 and transforming growth factor β (TGF-β) —when transgenically expressed in the pancreatic β-islets of NOD mice — prevented autoimmune diabetes [22,23]. The expression of downmodulatory cytokines in the target organ significantly reduced the diabetogenic potential of self-reactive T cells, so that splenocytes isolated from NOD mice transgenic for the human insulin promoter (ins) linked to IL-4 or TGF-β (ins–IL-4 or ins–TGF-β, respectively) were unable to transfer IDDM to NOD-*Scid* (immunodeficient) mice [23,24]. In addition, transgenic expression of IL-4 in the central nervous system protected SJL/J mice from EAE [25]. The release of these downmodulatory cytokines also seems to be involved in protection from autoimmunity mediated by regulatory T cell subsets. For example, oral tolerance against EAE achieved by feeding SJL/J mice

with the self antigen, myelin basic protein (MBP), appeared to be related to TGF-β secretion in MBP-specific T cell clones [26]. Additionally, regulatory T cell subsets derived either from the thymus ($CD4^+$ $CD8^-$) or the periphery ($CD4^+$ $CD45RC^-$) suppressed autoimmune diabetes by secreting IL-4 and TGF-β [27•]. These downmodulatory effects of IL-4 and TGF-β could be integral to direct inhibition of Th1, autoreactive T cell differentiation and activation [28,29].

Others have proposed that a shift of the cytokine profile of autoreactive T cells toward a Th2 phenotype could be responsible for protection from T-cell-mediated autoimmune diseases [23,30]. However, a recent report countered that proposal by stating that the same regulatory T cells involved in downregulation of IDDM inhibited antibody-mediated autoimmunity (thyroiditis) by secreting IL-4 and TGF-β [27•]. This led the authors to conclude that IL-4 and TGF-β could deter autoimmunity through direct suppression of autoreactive T cells.

Overall, these observations generally support the idea that Th1-inducing cytokines can directly upregulate T cell autoimmunity whereas cytokines that promote Th2 differentiation and, more importantly, downregulate Th1 autoimmune responses are invariantly associated with protection from autoimmune diseases. However, several exceptions to this widely believed paradigm have emerged recently.

The immunosuppressive role of proinflammatory cytokines

In many reports, investigators have pointed out that cytokines belonging to the inflammatory Th1 pathway could be critical to the counter-regulation of T-cell-mediated autoimmunity. There is now enough evidence that prolonged exposure to proinflammatory cytokines such as TNF can have anti-inflammatory and protective effects against T-cell-mediated autoimmunity [31••]. Despite the well-documented proinflammatory effect of TNF during early phases of immune responses, the late administration of this cytokine can protect NOD mice from IDDM: TNF is responsible for exacerbation of IDDM either when injected in NOD mice that are younger than 4 weeks [32] or when expressed transgenically in the pancreatic islets early in life [33•]; however the late, transgenic expression of TNF in the islets of NOD mice downmodulated the autoreactive T cell repertoire [34,35].

TNF also acted as a potent anti-inflammatory cytokine in autoimmune-mediated demyelination [36•]. Although there are numerous proofs for a suppressive role of TNF in T-cell-mediated autoimmunity, as yet the mechanisms underlying this regulation have not been fully clarified. The premise that TNF could tolerize autoreactive T cells was strongly reinforced by an elegant experiment in which TNF was expressed together with the *Leishmania major* antigen, LACK, in the pancreatic islets of mice carrying a transgenic LACK-specific TCR [37]. These mice responded poorly to LACK immunization and carried a reduced number of TCR-transgenic T cells with an activated phenotype. The nature of the tolerance that locally expressed TNF induced in the LACK-specific, autoreactive T cells was not defined. Although the cytokine profile of these autoreactive T cells shifted toward a Th2 type, this shift could be easily ascribed to a selective downmodulatory effect of TNF on Th1 cells.

Originally, TNF was thought to induce T cell apoptosis, providing a key pathway for the death of T cells so as to switch off unnecessary immune responses [38,39]. Another most intriguing hypothesis, which recently surfaced, pictured TNF as an immunosuppressor cytokine acting on T cells by attenuating TCR signal transduction pathways. Chronic stimulation with TNF appeared to impair early signaling events in T cells which could ultimately lead to defective propriocidal regulatory mechanisms (activation-induced death) among T cells activated at sites of inflammation [40].

IL-12 is another proinflammatory cytokine, critical for inducing Th1 differentiation, that has also downmodulated inflammatory autoimmune responses. In fact — even though chronic daily administration of exogenous IL-12 accelerated autoimmune diabetes in the NOD mice [9], as discussed earlier — intermittent administration of this proinflammatory cytokine resulted in protection from autoimmunity [41]. Furthermore, IL-12 treatment suppressed experimental autoimmune uveitis, most probably by hyperinduction of IFN-γ [42••].

Several reports in the literature describe a protective role for IFN-γ with respect to T-cell-mediated autoimmunity — thus supporting the theory that IFN-γ, much like TNF, can activate homeostatic mechanisms to control inflammatory responses. In particular, we have found that transgenic expression of IFN-γ in the pancreatic islets can actually reduce the diabetogenic potential of autoreactive T cells and prevent IDDM in streptozotocin-treated mice [43] as well as NOD mice (C King, E Jones, N Sarvetnick, unpublished data). On the other side, removal of IFN-γ is known to exacerbate T-cell-mediated autoimmune diseases such as EAE [44,45]. Interestingly, several regulatory T cell populations seem to require IFN-γ secretion to exert their downmodulatory effect on the pathogenesis of T-cell-mediated autoimmune diseases [46,47•]. The possible mechanisms responsible for IFN-γ-mediated downmodulation of inflammatory T cell responses are not entirely clear. It is well-documented that IFN-γ can have direct immunosuppressive effects on T cells [48–50]. In fact, evidence recently emerged that IFN-γ could protect from T-cell-mediated autoimmunity by direct suppression of autoreactive T cells [51]. Another possibility is that IFN-γ prevents autoimmunity by triggering Bcl-2-regulated apoptosis of autoreactive T cells [42••]. Alternatively,

IFN-γ could also induce cell death on effector autoimmune T cells activated in the absence of co-stimulatory signals [52].

From these studies, one clear-cut concept has emerged: proinflammatory cytokines secreted in large amounts late during the inflammation process can be critical to dampening T cell responses. Cytokines such as IL-12 and TNF may be required at an early time to induce autoimmunity by priming inflammatory Th1 responses; however, the late expression of the same cytokines could drive the terminal differentiation and death of T cells, including those engaged in autoreactive responses.

The foregoing findings clearly indicate that cytokines may have completely contradictory roles according to the time they enter the scene in the process of T-cell-mediated autoimmunity. In addition, cytokines may play an unexpected role in autoimmunity by modulating cellular populations other than T cells (e.g. APCs), as described in the section below.

Cytokines can modulate T-cell-mediated autoimmunity through their effect on APCs

Macrophages and dendritic cells (DCs) are believed to have a pivotal function early in the pathogenesis of T-cell-mediated autoimmune diseases [53,54]. F4/80$^+$ CD11b$^+$ macrophages and F4/80$^-$ CD11c$^+$ DCs appear in the early lymphocyte-rich infiltrates entering the pancreatic islets of diabetes-prone NOD mice and BB rats [55–57]. In a recent report the antigen-presenting function of macrophages and DCs has been clearly linked to the pathogenesis of autoimmune diabetes in NOD mice. That is, the early depletion of macrophages completely protected NOD mice from IDDM [58,59•]. These mice also lacked any autoreactive T cell response against islet antigens such as GAD65, suggesting that macrophages and DCs are important in priming the autoreactive T cell repertoire.

Although macrophages and DCs are considered major players in the early phases of autoimmune diseases, the events that trigger organ-specific autoimmunity remain mostly unknown. Apoptosis of few pancreatic β cells precedes insulitis in NOD mice [60,61]. Perhaps macrophages and DCs are essential for the early uptake and presentation of self antigens that follow the primary damage and apoptotic death of tissues [62••] during inflammatory processes. At the same time, macrophages could be critical for the activation of inflammatory, Th1, autoreactive cells by secreting IL-12 in the microenvironment — thereby driving the cytokine profile of self-reactive T cells toward a pathogenic Th1 type and activating autoreactive T cells with cytotoxic functions [63,64].

Many cytokines expressed in the target organs of disease can modulate autoimmunity by regulating both antigen-presenting and cytokine-secreting functions of macrophages and DCs. In particular, the early presence of classical inflammatory cytokines such as TNF and IFN-γ can activate autoreactive T cell responses by upregulating macrophage and DC functions. For example, production of TNF within the pancreatic islets accelerated IDDM in transgenic NOD mice that expressed TNF under the control of the rat insulin promoter (RIP) [33•]. In these mice, the pathogenic effect of TNF seemed to be mediated through an early recruitment and activation of DCs and macrophages — not by a direct damage to the pancreatic β cells. Moreover, the antigen-presenting function of these cell populations from the RIP–TNF NOD mice was more effective than that of the same cells from non-transgenic littermates. Thus, the presence of TNF in the islets favored the self-antigen uptake and presentation by macrophages and DCs.

IFN-γ is another strong inflammatory cytokine whose expression in the target organs leads to T-cell-mediated autoimmunity by strains of mice that are not genetically prone to autoimmune diseases [65]; in this model, the IFN-γ did not seem to upregulate autoimmunity by directly damaging the islets cells but rather by turning on an autoreactive T cell repertoire in Balb/c mice, which do not otherwise develop autoimmune diabetes. The diabetogenic effect of IFN-γ appeared to be mediated by activation of professional APCs. In fact, inhibition of macrophages through blockage of the type 3 complement receptor prevented the pathogenic signs of IDDM in transgenic ins–IFN-γ Balb/c mice [66]. Similarly, the transgenic expression of IFN-γ in the central nervous system of mice obtained under the control of the MBP promoter resulted in inflammatory T-cell-mediated diseases of the brain white matter — much like the autoimmune phenomena of multiple sclerosis in humans [67].

The mechanisms underlying IFN-γ-induced autoimmunity are still unclear; however many believe that IFN-γ can mediate the activation of autoreactive immune responses by increasing the uptake and presentation of self antigens on macrophages and DCs in target organs [68]. For example, IFN-γ can induce expression of MHC molecules on professional APCs [69] as well as cells that are targets of autoimmune destruction [70,71]. The transgenic expression of inflammatory cytokines could mimic the early events in the pathogenesis of T-cell-mediated autoimmune diseases. Inflammatory cytokines secreted during a tissue-specific infection can provide a signal sufficient for the activation of macrophages and DCs; such activated DC/macrophage precursors could then present self antigens to an autoreactive T cell repertoire of individuals who are predisposed to organ-specific autoimmunity [72••].

However, evidence indicates that cytokines of the Th2 type can also activate macrophages and DCs. An example came from BDC2.5–NOD mice, which have a transgenic TCR that recognizes an unknown islet-cell antigen [73]. Although these transgenic mice carry an enlarged autoreactive T cell repertoire, they do not develop autoimmunity

because their autoreactive naïve T cells do not receive an activation signal in the target organ [74]. However, the transgenic expression of IL-4 in the pancreatic islets of these BDC2.5 mice was sufficient to reverse that status and induce IDDM [24]. We have recently found that IL-4 can trigger the activation of the BDC2.5 T cell clone by increasing the islet-antigen-presenting function of DCs and macrophages (M Falcone, N Sarvetnick, unpublished data). The classical inflammatory pathway — characterized by secretion of inflammatory cytokines IL-1, TNF and free radicals — is induced in macrophages by IFN-γ and inhibited by IL-4 [69]. However, nonclassical inflammatory cytokines such as IL-4 can induce the alternative pathway of macrophage activation and enhance their capacity for phagocytosis as well as increase expression levels of MHC class II molecules [75]. Moreover, IL-4 is known to induce DC maturation in conjunction with GM-CSF [76]. Both these effects of IL-4 on DC/macrophage APCs could trigger autoimmunity by inducing self-antigen uptake and presentation of the self antigen in the islets of BDC2.5–IL-4-transgenic NOD mice.

This recent finding provides further evidence that cytokines mediate multiple effects on the pathogenesis of T-cell-mediated autoimmunity, depending on the phase of an immune response and the cell population with which they interact. In the case of IL-4, which has a direct down-modulatory effect on the differentiation of autoreactive Th1 cells, this cytokine may operate through its action on the DCs and macrophages that present self antigen to the already established T cell repertoire in BDC2.5-transgenic mice. On the other hand, late in the course of inflammatory immune responses, Th1 cytokines (such as IFN-γ), whose early expression could be sufficient to generate tissue-specific autoimmunity, exert control via APCs. We mentioned earlier that IFN-γ can effectively limit Th1 immune responses by directly downmodulating T cells. However, IFN-γ was also found to be responsible for macrophage deactivation through upregulation of the A_{2b} adenoside receptor [77•]. Stimulation of the A_{2b} receptor by adenoside and its analogs led to decreased expression of MHC class II genes as well as to decreased production of nitric oxide synthase and proinflammatory cytokines. The final outcome of this mechanism of negative feed-back could be the downmodulation of both the antigen-presenting function and the inflammatory phenomena displayed by macrophages in the course of autoimmune responses and in prevention of destructive autoimmunity.

Conclusions

The large number of studies performed to analyze interactions between cytokines and autoimmune responses indicate the extreme complexity of the cytokine network. Consequently, the regulation that cytokines superimpose on autoimmunity is a finely tuned balance between activation and downmodulation of an individual autoreactive T cell repertoire. Factors such as the duration of cytokine exposure and the type of cell population involved strongly influence that balance.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Wucherpfennig KW, Strominger JL: Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* 1995, 80:695-705.
2. Libleau RS, Singer SM, McDevitt HO: Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* 1995, 16:34-38.
3. Tian J, Olcott AP, Hanssen LR, Zekzer D, Middleton B, Kaufman DL: Infectious Th1 and Th2 autoimmunity in diabetes-prone mice. *Immunol Rev* 1998, 164:119-127.
4. Seder R, Gazzinelli R, Sher A, Paul W: Interleukin-12 acts directly on CD4+ T cells to enhance priming for IFN-γ production and diminishes interleukin-4 inhibition of such priming. *Proc Natl Acad Sci USA* 1993, 90:10188-10192.
5. Manetti R, Parronchi P, Giudizi M, Piccinni M, Maggi E, Trinchieri G, Romagnani S: Natural killer cell stimulator factor (interleukin-12) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 1993, 177:1199-1204.
6. Magram J, Connaughton S, Warrier R, Carvajal D, Wu C, Ferrante J, Stewart C, Sarmiento U, Faherty D, Gately M: IL-12-deficient mice are defective in IFN-gamma production and type 1 cytokine responses. *Immunity* 1996, 4:471-481.
7. Trembleau S, Germann T, Gately MK, Adorini L: The role of IL-12 in the induction of organ-specific autoimmune diseases. *Immunol Today* 1995, 16:383-386.
8. Caspi RR: IL-12 in autoimmunity. *Clin Immunol Immunopathol* 1998, 88:4-13.
9. Trembleau S, Penna G, Bosi E, Mortara A, Gately MK, Adorini L: Interleukin 12 administration induces T helper type 1 cells and accelerates autoimmune diabetes in NOD mice. *J Exp Med* 1995, 181:817-821.
10. Leonard JP, Waldburger KE, Schaub RG, Smith T, Hewson AK, Cutner ML, Goldman SJ: Regulation of the inflammatory response in animal models of multiple sclerosis by interleukin-12. *Crit Rev Immunol* 1997, 17:545-553.
11. Rothe H, O'Hara R, Martin S, Kolb H: Suppression of cyclophosphamide induced diabetes development and pancreatic Th1 reactivity in NOD mice treated with the interleukin-12 antagonist IL-12 (p40)². *Diabetologia* 1997, 40:841-846.
12. Trembleau S, Penna G, Gregori S, Gately MK, Adorini L: Deviation of pancreas-infiltrating cells to Th2 by interleukin-12 antagonist administration inhibits autoimmune diabetes. *Eur J Immunol* 1997, 27:2330-2339.
13. Leonard JP, Waldburger KE, Goldman SJ: Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. *J Exp Med* 1995, 181:381-386.
14. Constantinescu CS, Wysocka M, Hilliard B, Ventura ES, Lavi E, Trinchieri G, Rostami A: Antibodies against IL-12 prevent superantigen-induced and spontaneous relapses of experimental autoimmune encephalomyelitis. *J Immunol* 1998, 161:5097-5104.
15. Yokoi H, Kato K, Kezuka T, Sakai J, Usui M, Yagita H, Okumura K: Prevention of experimental autoimmune uveoretinitis by monoclonal antibody to interleukin-12. *Eur J Immunol* 1997, 27:641-646.
16. Tarrant TK, Silver PB, Chan CC, Wiggert B, Caspi RR: Endogenous IL-12 is required for induction and expression of experimental autoimmune uveitis. *J Immunol* 1998, 161:122-127.
17. McIntyre KW, Shuster DJ, Gillooly KM, Warrier RR, Connaughton SE, Hall LB, Arp LH, Gately MK, Magram J: Reduced incidence and severity of collagen-induced arthritis in interleukin-12-deficient mice. *Eur J Immunol* 1996, 26:2933-2938.

18. Trinchieri G: Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu Rev Immunol* 1995, 13:251-276.

19. Segal BM, Klinman DM, Shevach EM: Microbial products induce autoimmune disease by an IL-12-dependent pathway. *J Immunol* 1997, 158:5087-5090.

20. Segal BM, Shevach EM: IL-12 unmasks latent autoimmune disease in resistant mice. *J Exp Med* 1996, 184:771-775.

21. •• Chang JT, Shevach EM, Segal BM: Regulation of interleukin (IL)-12 receptor beta2 subunit expression by endogenous IL-12: a critical step in the differentiation of pathogenic autoreactive T cells. *J Exp Med* 1999, 189:969-978.
This study introduces a new and intriguing hypothesis that genetic predisposition to autoimmunity could be linked to IL-12 secretion in the absence of infectious agents. The authors investigated the T-cell-induced IL-12 secretion in two strains of mice, SJL/J and B10.S, that are respectively highly susceptible and resistant to EAE. The results indicate that, in the SJL/J mice, T cells specific for MBP as well as for a foreign antigen were able to induce IL-12 secretion by APCs and IL-12-mediated upregulation of the IL-12Rβ2 subunit on the T cells through interaction of CD40 and CD40-ligand (CD40L). Conversely, in a resistant strain – such as B10.S mice – self-reactive T cells did not express the CD40L and were not able to upregulate the IL-12Rβ2 subunit unless exogenous IL-12 was added to the cultures. The presence of the IL-12Rβ2 subunit on autoreactive T cells was found to correlate with their encephalitogenic potential. Therefore, the ability of autoreactive T cells to induce IL-12 secretion and their own Th1 differentiation could predispose autoimmune-prone strains of mice to the activation of unnecessary inflammatory responses and, ultimately, to destructive autoimmunity.

22. Mueller R, Krahl T, Sarvetnick N: Pancreatic expression of interleukin-4 abrogates insulitis and autoimmune diabetes in nonobese diabetic (NOD) mice. *J Exp Med* 1996, 184:1093-1099.

23. King C, Davies J, Mueller R, Lee MS, Krahl T, Yeung B, O'Connor E, Sarvetnick N: TGF-beta1 alters APC preference, polarizing islet antigen responses toward a Th2 phenotype. *Immunity* 1998, 8:601-613.

24. Mueller R, Bradley LM, Krahl T, Sarvetnick N: Mechanism underlying counterregulation of autoimmune diabetes by IL-4. *Immunity* 1997, 7:411-418.

25. Furlan R, Poliani PL, Galbiati F, Bergami A, Grimaldi LM, Comi G, Adorini L, Martino G: Central nervous system delivery of interleukin 4 by a nonreplicative herpes simplex type 1 viral vector ameliorates autoimmune demyelination. *Hum Gene Ther* 1998, 9:2605-2617.

26. Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL: Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994, 265:1237-1240.

27. • Seddon B, Mason D: Regulatory T cells in the control of autoimmunity: the essential role of transforming growth factor beta and interleukin 4 in the prevention of autoimmune thyroiditis in rats by peripheral CD4(+)CD45RC- cells and CD4(+)CD8(-) thymocytes. *J Exp Med* 1999, 189:279-288.
In this study the mechanisms underlying T-cell-mediated protection from autoimmunity are analyzed. Subsets of $CD4^+$ $CD8^-$ thymocytes as well as of peripheral CD4+ CD45RC- T cells are known to have downmodulatory potential with respect to T-cell-mediated autoimmune diseases by releasing immunosuppressive cytokines such as IL-4 and TGF-β. In this article, the results demonstrate that the same T cell subsets could also downmodulate antibody-mediated thyroiditis induced by thymectomy followed by irradiation (TsX protocol). Prevention of thyroiditis either by $CD4^+$ $CD8^-$ thymocytes or peripheral CD4+ CD45RC- T cells was abolished by inhibition of either IL-4 or TGF-β activity. These results clearly suggest that cytokines that are downmodulatory for the Th1 pathway could also have an immunosuppressive quality for antibody-mediated autoimmune diseases. The authors' conclusions reinforce the view that the downmodulatory effect of IL-4 and TGF-β on autoimmunity stems from a direct immunosuppression of autoreactive T cells rather than a shift toward a Th2 phenotype.

28. Seder RA, Paul W, Davis M, Fazekas de St Groth B: The presence of interleukin-4 during in vitro priming determines the lymphokine-producing potential of CD4+ T cells from T cell receptor transgenic mice. *J Exp Med* 1992, 176:1091-1098.

29. Shull M, Ormsby I, Kier A, Pawlowski S, Diebold R, Yin M, Allen R, Sidman C, Proetzel G, Calvin D: Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992, 359:693-699.

30. Gallichan WS, Balasa B, Davies JD, Sarvetnick N: Pancreatic IL-4 expression results in islet-reactive Th2 cells that inhibit diabetogenic lymphocytes in the nonobese diabetic mouse. *J Immunol* 1999, 163:1698-1708.

31. •• Copa AP: Regulation of autoimmunity by proinflammatory cytokines. *Curr Opin Immunol* 1998, 10:669-676.
The author of this review carefully analyzes the extensive evidence that TNF has a protective role in T-cell-mediated autoimmunity. The review includes a detailed list of experimental models that use either TNF-transgenic or TNF-treated mice as well as TNF-knockout and TNF-depleted mice to point out a clear-cut critical role for this inflammatory cytokine in downmodulating inflammatory autoimmune diseases. Moreover, the possible mechanisms underlying TNF-mediated immunosuppression are discussed as well as the most recent experiments, both *in vivo* and *in vitro*, implicating a direct immunomodulatory effect of TNF on the TCR signaling pathway.

32. Yang XD, Tisch R, Singer SM, Cao ZA, Liblau RS, Schreiber D, McDevitt HO: Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J Exp Med* 1994, 180:995-1004.

33. • Green EA, Eynon EE, Flavell RA: Local expression of TNFalpha in neonatal NOD mice promotes diabetes by enhancing presentation of islet antigens. *Immunity* 1998, 9:733-743.
Islet-specific expression of TNF in neonatal NOD mice under the control of RIP accelerated autoimmune diabetes by activating APCs such as macrophages and DCs. $F4/80^+$ $CD11b^+$ macrophages and $F4/80^-$ $CD11c^+$ DCs were visible in the islets of RIP–TNF NOD mice that were only 10 days old. In these transgenic mice, macrophages and DCs were activated and increased their expression of co-stimulatory molecules such as CD86 and MHC class II molecules. Consequently, macrophages and DCs of transgenic RIP–TNF NOD mice demonstrated an increased ability to activate autoreactive T cells *in vitro*, even if the islet self-antigen was not added to the *in vitro* T cell cultures. This observation suggested that the presence of TNF *in vivo* in the islets could favor self-antigen uptake by macrophages and DCs. Additionally, the degree of maturation of DCs was increased in the RIP–TNF NOD mice and the authors proposed that the early presence of a strong inflammatory cytokine such as TNF in the pancreatic islets could promote IDDM pathogenesis by inducing DC maturation *in situ*.

34. Grewal I, Grewal K, Wong F, Picarella D, Janeway C Jr, Flavell R: Local expression of transgene encoded TNFα in islets prevents autoimmune diabetes in nonobese diabetic (NOD) mice by preventing the development of auto-reactive islet-specific T cells. *J Exp Med* 1996, 184:1983-1974.

35. Hunger RE, Carnaud C, Garcia I, Vassalli P, Mueller C: Prevention of autoimmune diabetes mellitus in NOD mice by transgenic expression of soluble tumor necrosis factor receptor p55. *Eur J Immunol* 1997, 27:255-261.

36. • Liu J, Marino MW, Wong G, Grail D, Dunn A, Bettadapura J, Slavin AJ, Old L, Bernard CC: TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. *Nat Med* 1998, 4:78-83.
This report shows that lack of TNF in congenitally deficient mice predisposes to autoimmunity. B6 mice are susceptible to autoimmune demyelination induced by immunization with the myelin oligodendrocyte glycoprotein (MOG) whereas the 129 strain is completely resistant. TNF gene ablation rendered 129 mice susceptible to MOG-induced disease and also increased both clinical and hystopathological signs of inflammation and demyelination in the B6 strain of mice. Conversely, systemic administration of recombinant TNF significantly ameliorated autoimmune demyelination in these mice.

37. McSorley SJ, Soldera S, Malherbe L, Carnaud C, Lockaley RM, Flavell RA, Glaichenhaus N: Immunological tolerance to a pancreatic antigen as a result of local expression of TNFalpha by islet beta cells. *Immunity* 1997, 7:401-409.

38. Zhang L, Fisher G, Miller R, Perchon J, Lynch D, Lenardo M: Induction of apoptosis in mature T cells by tumor necrosis factor. *Nature* 1995, 377:348-351.

39. Sytwu H, Liblau R, McDevitt H: The roles of Fas/APO-1 (CD95) and TNF in antigen-induced programmed cell death in T cell receptor transgenic mice. *Immunity* 1996, 5:17-30.

40. Copa AP, Liblau RS, Yang XD, Congia M, Laudanna C, Schreiber RD, Probert L, Kollias G, McDevitt HO: Chronic tumor necrosis factor alters T cell responses by attenuating T cell receptor signaling. *J Exp Med* 1997, 185:1573-1584.

41. O'Hara R, Henderson S, Nagelin A: Prevention of a Th1 disease by a Th1 cytokine: IL-12 and diabetes in NOD mice. *Ann N Y Acad Sci* 1996, 795:241-249.

42. Tarrant TK, Silver PB, Wahlsten JL, Rizzo LV, Chan CC, Wiggert B, Caspi RR: Interleukin 12 protects from a T helper type 1-mediated autoimmune disease, experimental autoimmune uveitis, through a mechanism involving interferon gamma, nitric oxide, and apoptosis. *J Exp Med* 1999, 189:219-230.

This study showed that IL-12 could unexpectedly protect against a Th1-mediated autoimmune disease such as experimental autoimmune uveitis. Interestingly, the protection from autoimmune uveitis was abolished in IFN-γ deficient mice – suggesting that IL-12-mediated regulation of autoimmunity was obtained through IFN-γ secretion. The authors provided interesting evidence that explains possible mechanisms underlying immunoregulation mediated by proinflammatory cytokines. IL-12-treated mice had a reduced number of cells in the draining lymph nodes and reduced response to the self-antigen *in vitro*. Moreover, the number of apoptotic cells was increased in the draining lymph nodes of mice that received IL-12 at the time of priming with self-antigen. These findings, together with the observation that protection was lacking in Bcl-2-deficient mice, supported the hypothesis that IFN-γ hyperproduction induced by IL-12 could trigger Bcl-2-controlled apoptotic deletion of antigen-specific T cells. Since the protection was lost in mice deficient in iNOS (inducible nitric oxide synthase) the authors suggested that upregulation of iNOS and production of NO might also be required to trigger apoptosis of autoreactive T cells.

43. Gu D, Amuah M, Sawyer S, Sarvetnick N: Transgenic mice expressing IFN-gamma in pancreatic beta-cells are resistant to streptozotocin-induced diabetes. *Am J Physiol* 1995, 269:1089-1094.

44. Krakowski M, Owens T: Interferon-gamma confers resistance to experimental allergic encephalomyelitis. *Eur J Immunol* 1996, 26:1641-1646.

45. Willenborg DO, Fordham S, Bernard CC, Cowden WB, Ramshaw IA: IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J Immunol* 1996, 157:3223-3227.

46. Kumar V, Sercarz E: Induction or protection from experimental autoimmune encephalomyelitis depends on the cytokine secretion profile of TCR peptide-specific regulatory CD4 T cells. *J Immunol* 1998, 161:6585-6591.

47. • Falcone M, Yeung B, Tucker L, Rodriguez E, Sarvetnick N: A defect in IL-12-induced activation and IFN-γ secretion of peripheral natural killer T cells in nonobese diabetic mice suggests new pathogenic mechanisms for insulin-dependent diabetes mellitus (IDDM). *J Exp Med* 1999, 190:963-972.

NKT cells have been shown to mediate immunoregulation of T-cell-mediated autoimmunity. Their defect in many autoimmune-prone strains of mice represents key evidence that they play a critical role to balance T cell responses between protective immunity and autoimmunity. In this study we have shown that the defect of the NKT cell subset in NOD mice specifically involved their IL-12-induced activation as well as IFN-γ secretion. These results provided strong evidence that a defect of the Th1 pathway, involving the NKT cell subset, could be associated with autoimmunity in NOD mice that develop spontaneous IDDM.

48. Holda J, Maier T, Claman H: Natural suppressor activity in graft-vs-host spleen and normal bone marrow is augmented by IL-2 and IFN-γ. *J Immunol* 1986, 137:3536-3543.

49. Holda J, Maier T, Claman H: Evidence that IFN-γ is responsible for natural suppressor activity in GVHD spleen and normal bone marrow. *Transplantation* 1988, 45:772-777.

50. Huchet R, Bruley-Rosset M, Mathiot C, Grandjon D, Halle-Pannenko O: Involvement of interferon-γ and transforming growth factor-β in graft-vs-host reaction-associated immunosuppression. *J Immunol* 1993, 150:2517-2524.

51. Meyers CM, Zhang Y: Immunomodulatory effects of interferon gamma on autoreactive nephritogenic T-cell clones. *Kidney Int* 1999, 55:1995-1408.

52. Liu Y, Janeway C: Interferon-γ plays a critical role in induced cell death of effector T cells: a possible third mechanism of self-tolerance. *J Exp Med* 1990, 172:1735-1739.

53. Ludewig B, Odermatt B, Landmann S, Hengartner H, Zinkernagel RM: Dendritic cells induce autoimmune diabetes and maintain disease via de novo formation of local lymphoid tissue. *J Exp Med* 1998, 188:1493-1501.

54. Yoon JW, Jun HS, Santamaria P: Cellular and molecular mechanisms for the initiation and progression of beta cell destruction resulting from the collaboration between macrophages and T cells. *Autoimmunity* 1998, 27:109-122.

55. Lee KU, Kim MK, Amano K, Pak CY, Jaworski MA, Mehta JG, Yoon JW: Preferential infiltration of macrophages during early stages of insulitis in diabetes-prone BB rats. *Diabetes* 1988, 37:1053-1058.

56. Walker R, Bone A, Cooke A, Baird J: Distinct macrophage subpopulations in pancreas of pre-diabetic BB/E rats: possible role for macrophages in the pathogenesis of IDDM. *Diabetes* 1988, 37:1623-1629.

57. Jansen A, Homo-Delarche R, Hodiljkaas H, Leenen P, Dardenne M, Drexhage H: Immunohistochemical characterization of monocyte-macrophages and dendritic cells involved in the initiation of insulitis and beta-cell destruction in NOD mice. *Diabetes* 1994, 43:667-675.

58. Lee KI, Amano K, Yoon JW: Evidence for initial involvement of macrophages in development of insulitis in NOD mice. *Diabetes* 1988, 37:989-991.

59. • Jun H, Yoon CS, Zbytnuik L, van Rooijen N, Yoon JW: The role of macrophages in T cell-mediated autoimmune diabetes in nonobese diabetic mice. *J Exp Med* 1999, 189:347-358.

Autoimmune diabetes could be blocked in NOD mice by depletion of macrophages through treatment with a mixture of liposomes containing phosphatidylin choline (lip-Cl_2MDP). Interestingly, the absence of macrophages in the microenvironment inhibited the generation of a pathogenic autoimmune T cell repertoire. Splenocytes from macrophage-depleted NOD mice could not transfer diabetes to NOD-*Scid* mice and splenocytes responded less strongly to *in vitro* stimulation with self antigens such as islet-cell antigen and GAD65. The effect could be integrally ascribed to the inhibition of antigen-presenting function and IL-12 secretion by macrophages. As a proof, IDDM could be restored in lip-Cl_2MDP-treated NOD mice by IL-12 injection.

60. Chervonsky A, Wang Y, Wong FS, Visintin I, Flavell RA, Janeway CA Jr, Matis LA: The role of Fas in autoimmune diabetes. *Cell* 1997, 89:17-24.

61. Itoh N, Imagawa A, Hanafusa T, Waguri M, Yamamoto K, Iwashashi H, Moriwaki M, Nakajima H, Miyagawa J, Namba M *et al.*: Requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice. *J Exp Med* 1997, 186:613-618.

62. • Rovere P, Vallinoto C, Bondanza A, Crosti MC, Rescigno M, Ricciardi-Castagnoli P, Rugarli C, Manfredi AA: Bystander apoptosis triggers dendritic cell maturation and antigen-presenting function. *J Immunol* 1998, 161:4467-4471.

The effect of apoptotic cells on DC activation and maturation was evaluated by studying the *in vitro* interaction between murine RMA lymphoma cells committed to apoptosis by UV irradiation and an immature DC line (D1). The results demonstrated that apoptotic cells, internalized by DCs, increased class-I- and class-II-mediated antigen-presentation as well as secretion of proinflammatory cytokines by DCs in a dose-dependent fashion.

63. Trinchieri G: IL-12 and its role in generation of Th1 cells. *Immunol Today* 1993, 14:335-337.

64. Kennedy M, Picha K, Shanebeck K, Anderson D, Grabstein K: Interleukin-12 regulates the proliferation of Th1, but not Th2 or Th0 clones. *Eur J Immunol* 1994, 24:2271-2278.

65. Sarvetnick N, Shizuru J, Liggitt D, Martin L, McIntyre B, Gregory A, Parslow T, Stewart T: Loss of pancreatic islet tolerance induced by beta-cell expression of interferon-gamma. *Nature* 1990, 346:844-847.

66. Gu D, O'Reilly L, Molony L, Cooke A, Sarvetnick N: The role of infiltrating macrophages in islet destruction and regrowth in a transgenic model. *J Autoimmun* 1995, 8:483-492.

67. Horwitz MS, Evans CF, McGavern DB, Rodriguez M, Oldstone MBA: Primary demyelination in transgenic mice expressing interferon-gamma. *Nat Med* 1997, 3:1037-1041.

68. Campbell IL, Oxbrow L, Koulmanda M, Harrison LC: IFN-γ induces islet cell MHC antigens and enhances autoimmune, streptozotocin-induced diabetes in the mouse. *J Immunol* 1988, 140:1111-1116.

69. Cao H, Wolff RG, Meltzer MS, Crawford RM: Differential regulation of class II MHC determinants on macrophages by IFN-gamma and IL-4. *J Immunol* 1989, 143:3524-3531.

70. Campbell IL, Wong G, Schrader J, Harrison L: **Interferon-gamma enhances the expression of the major histocompatibility class I antigens on mouse pancreatic beta cells.** *Diabetes* 1985, 34:1205-1209.

71. Bergsteindottir K, Brennan A, Jessen K, Mirsky R: **In the presence of dexamethasone, gamma interferon induces rat oligodendrocytes to express major histocompatibility complex class II molecules.** *Proc Natl Acad Sci USA* 1992, 89:9054-9058.

72. Horwitz MS, Bradley L, Harbertson J, Krahl T, Lee J, Sarvetnick N: **Diabetes induced by coxsackie virus: initiation by bystander damage and not molecular mimicry.** *Nat Med* 1998, 4:781-785.

This article provides an extremely elegant two-phase model for the pathogenesis of T-cell-mediated autoimmune diseases. Coxsackie infection did not lead to total destruction of pancreatic islets and diabetes in NOD mice. However, the presence of coxsackie virus in the islets could induce the necessary bystander stimulus to activate the target tissue and present self antigens to the autoreactive T cell repertoire of BDC2.5 mice that carry a transgenic TCR specific for an islet antigen. The observation that the tissue-specific viral infection could trigger autoimmunity only in mice with an enlarged autoimmune T cell repertoire suggested that the pathogenesis of T-cell-mediated autoimmunity is characterized by two different phases. Amplification of the autoimmune T cell repertoire could happen in the periphery and be largely dependent on the genetic background of each individual. In addition, self-antigen-presentation and recruitment of autoreactive T cells in the target tissue through infection-induced bystander activation of APCs could represent a second signal that is required to trigger organ-specific autoimmunity.

73. Katz JD, Wang B, Haskins K, Benoist C, Mathis D: **Following a diabetogenic T cell from genesis through pathogenesis.** *Cell* 1993, 74:1089-1100.

74. Andre-Schmutz I, Hindelang C, Benoist C, Mathis D: **Cellular and molecular changes accompanying the progression from insulitis to diabetes.** *Eur J Immunol* 1999, 29:245-255.

75. Goerdt S, Orfanos CE: **Other functions, other genes: alternative activation of antigen-presenting cells.** *Immunity* 1999, 10:137-142.

76. Masurier C, Pioche-Durieu C, Colombo B, Lacave R, Lemoine F, Klatzmann D, Guigon M: **Immunophenotypical and functional heterogeneity of dendritic cells generated from murine bone marrow cultured with different cytokine combinations: implications for anti-tumoral cell therapy.** *Immunology* 1999, 96:569-577.

77. Xaus J, Mirabet M, Lloberas J, Soler C, Lluis C, Franco R, Celada A: **IFN-gamma up-regulates the A_{2b} adenosine receptor expression in macrophages: a mechanism of macrophage deactivation.** *J Immunol* 1999, 162:3607-3614.

This report describes an interesting mechanism of negative feed-back played by IFN-γ on macrophages. Adenosine is an anti-inflammatory molecule that downregulates both antigen-presenting function and cytokine secretion in macrophages. In this study IFN-γ upregulated the expression of the A_{2b} adenosine receptor, thus rendering macrophages more susceptible to the anti-inflammatory action of adenosine. Among the macrophage functions inhibited by adenosine there was also IFN-γ-induced expression of MHC class II genes and that of nitric oxide synthase and proinflammatory cytokines. These findings lead us to conclude the IFN-γ-mediated upregulation of A_{2b} receptor could represent a feed-back mechanism to downregulate macrophage activation and, in general, to control inflammatory immune responses.

EXHIBIT "D"

Protein kinase C mediates experimental colitis in the rat

JAMES F. BROWN, QING CHANG, BRIAN D. SOPER, AND BARRY L. TEPPERMAN
Department of Physiology, Faculty of Medicine,
University of Western Ontario, London, Ontario, Canada N6A 5C1

Brown, James F., Qing Chang, Brian D. Soper, and Barry L. Tepperman. Protein kinase C mediates experimental colitis in the rat. *Am. J. Physiol.* 276 (*Gastrointest. Liver Physiol.* 39): G583–G590, 1999.—Protein kinase C (PKC) plays an important role in the cell signal transduction of many physiological processes. In contrast to these physiological responses, increases in PKC activity have also been associated with inflammatory disease states, including ulcerative colitis. The objective of this study was to examine the role of PKC as a causative mediator in initiation of experimentally induced colitis in the rat. Colitis was induced in rats by intrarectal (0.6 ml) instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS; 75 mg/kg in 50% ethanol) or the PKC activator phorbol 12-myristate 13-acetate (PMA; 1.5–3.0 mg/kg in 20% ethanol). Gross and histological mucosal damage, mucosal neutrophil infiltration, mucosal PKC activity, and PKC protein content for PKC isoforms α, β, δ, and ε were assessed 2 h to 14 days after an inflammatory challenge. Both PKC activity and mucosal injury increased significantly within 4 h of TNBS treatment. PKC activity was maximal at 7 days and declined at 14 days, whereas mucosal damage became maximal at 1 day and declined after 7 days. In contrast, neutrophil infiltration as assessed by myeloperoxidase activity only increased 12 h after TNBS treatment, became maximal 1 day after TNBS administration, and declined thereafter. PKCβ, -δ, and -ε were increased in response to TNBS, whereas PKCα protein content was decreased. The PKC antagonists staurosporine and GF-109203X (25 ng/kg iv) reduced TNBS-induced changes in mucosal PKC activity and the degree of mucosal damage. In contrast, neutropenia induced by antineutrophil serum treatment did not significantly affect the degree of injury or mucosal PKC activity. Furthermore, activation of mucosal PKC activity with PMA also induced mucosal damage, which was also inhibited by pretreatment with a PKC antagonist. In conclusion, these results suggest that increases in PKC activity play a causative role in TNBS-induced colitis. The PKC-mediated response to TNBS does not appear to involve neutrophil infiltration.

2,4,6-trinitrobenzenesulfonic acid; inflammation; phorbol 12-myristate 13-acetate; myeloperoxidase protein kinase C antagonist

PROTEIN KINASE C (PKC) plays an important role in many signaling pathways, including gene expression, cell growth and differentiation, secretion of hormones and neurotransmitters, and membrane functions (24). The signal transduction of these functions is achieved through a variety of isoforms of PKC (23–25).

In addition to its many physiological roles, PKC has also been associated with inflammatory disease states. Activation of PKC activity is associated with inflammation in a number of tissues, including skin, cartilage, and epithelial cells of the airway and glomerulus (10, 31, 35, 36). PKC has also been shown to play a role in the sensitization of endothelial cells to bacterial endotoxin challenge (20). PKC activation has also been implicated in bile salt-mediated injury to hepatocytes (16) and radiation-induced apoptosis of thymocytes (44). Similarly, PKC activity has been shown to be elevated in a variety of cell types in response to a number of inflammatory challenges, including treatment with platelet-activating factor, cytokines, and exposure to oxidant stress (4, 15, 17, 30, 41). PKC also regulates a variety of signal transduction events implicated in the pathogenesis of inflammation, including the biosynthesis of nitric oxide, inflammatory cytokines, superoxide production, and the activation of phospholipase A_2 (14, 34).

In the intestine, PKC activity was found to be elevated in biopsy samples taken from patients with ulcerative colitis (33). PKC activation has also been shown to increase intestinal endothelial and epithelial permeability in vivo and in vitro (3, 27, 29). Furthermore, intraluminal instillation of PKC activators such as phorbol 12-myristate 13-acetate (PMA) has been shown to result in ileal and colonic injury in experimental animals (9, 28). However, the primary nature of PKC activation in experimentally induced intestinal mucosal injury is unknown.

In the present study, we examine the role of PKC as a causative mediator in the initiation of colonic mucosal damage in response to intraluminal instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS). In addition, we examine whether PKC plays a critical role in PMA-induced injury in the rat colon, and we also examine the role of neutrophils in the inflammation produced by activation of PKC activity.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 180–200 g were purchased from Canada Breeding Labs (St. Constant, PQ) and were maintained in a temperature-controlled environment (22°C) on a 12:12-h light-dark cycle with standard laboratory chow and tap water available ad libitum. All studies were approved by the University of Western Ontario Animal Care Committee, and all animals were treated according to the guidelines set out by the Canadian Council on Animal Care.

Treatments

Induction of colonic inflammation. The rats were randomized into treatment groups, and colitis was induced using two methods. First, colitis was induced with TNBS (75 mg/kg in 50% ethanol), according to a modified version of a method described by Morris et al. (22). Second, colitis was induced

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

with intraluminal instillation of PMA (1.5–3.0 mg/kg in 20% ethanol), according to a method previously described by Fretland et al. (9). In some studies, the negative control compound for PMA, 4α-phorbol 12-myristate 13-acetate (4α-PMA) (1), was used in the concentration of 3.0 mg/kg. Briefly, in these studies, a rubber catheter was inserted rectally into the colon, such that the tip was ~8 cm proximal to the anus. A volume of 0.6 ml of either TNBS, PMA, or 4α-PMA was instilled into the lumen of the colon through the catheter. Control animals received either 0.6 ml of 20% ethanol, 50% ethanol, or 0.9% saline, administered as described above. Vehicle- and TNBS- or PMA-treated animals were always housed in separate cages.

Drugs and agents. Before induction of colitis, animals were subdivided to receive the following treatments: *1*) antineutrophil serum (ANS; Accurate Chemical and Scientific; 100 μl ip; 2 h before induction of colitis) at a dose that has previously been demonstrated to reduce circulating neutrophil concentrations to <5% of control concentrations within 2 h of injection (38), *2*) the PKC antagonists staurosporine or bisindolylmaleimide I (GF-109203X; Biomol, Plymouth Meeting, PA; 25 ng/kg iv; 30 min before induction of colitis). The doses of these antagonists have previously been used to inhibit PKC activity in vivo (21, 26).

Assessment of Colonic Injury and PKC Activity

Tissue damage. The colon was removed under sodium pentobarbital anesthesia, opened by longitudinal incision, rinsed under tap water, and pinned to an "ice-cold" wax block. Slide photographs were taken, and damage to the colon was assessed macroscopically by three naive observers using criteria described by Bell et al. (2). The mean of the three individual assessments was used for statistical comparisons. Immediately after the samples were photographed, the mucosa was removed by scraping with a blunt spatula. Samples were snap frozen in liquid nitrogen for later determination of myeloperoxidase and PKC activities.

In some studies, tissues from TNBS-treated rats were fixed in Formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin and were examined by light microscopy. The thickness of colonic wall was determined using a micrometer by measuring the distance from the serosal surface to the luminal surface of the mucosa. Measurements were done in triplicate.

Measurement of myeloperoxidase activity. Mucosal myeloperoxidase (MPO) levels were measured to provide an index of polymorphonuclear leukocyte infiltration. MPO activity was determined as described by Wallace (10). Briefly, frozen samples from mucosal scrapings were suspended in 50 mM phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (pH 6.0; Sigma) at a tissue concentration of 50 mg/ml. Samples were homogenized three times for 30 s each, frozen and thawed three times in an acetone-dry ice bath, and centrifuged at 40,000 *g* for 15 min at 4°C. MPO activity in the supernatant was determined by adding 100 μl of the supernatant to 2.9 ml of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/ml *o*-dianisidine hydrochloride (Sigma) and 0.0005% wt/vol hydrogen peroxide. The change in absorbance at 460 nm over a 3-min period was measured. MPO activity is presented as moles of hydrogen peroxide converted to water in 1 min at 22°C.

Measurement of PKC activity. Frozen mucosal scrapes were resuspended in 50 mM Tris·HCl buffer (pH 7.4) containing 5 mM EDTA, 10 mM EGTA, 50 μg/ml phenylmethylsulfonyl fluoride (PMSF), 10 mM benzamidine, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 0.3% wt/vol β-mercaptoethanol, and 10 mM okadaic acid. Tissue was homogenized at full speed on ice in an Ultra-Turrax tissue homogenizer for 15 s. A 25-μl aliquot was removed for determination of PKC activity using a commercially available kit (RPN 77, Amersham International) that measures the transfer of [γ-^{32}P]ATP to a peptide specific for PKC.

Measurement of PKC Protein Content

Materials. Affinity-purified rabbit polyclonal anti-PKCα, anti-PKCβ$_{II}$, anti-PKCδ, and anti-PKCε were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The secondary antibody was a goat anti-rabbit antibody conjugated to horseradish peroxidase (HRP) from Amersham. Rainbow electrophoresis molecular weight marker, the enhanced chemiluminescence (ECL) kit, Hybond ECL nitrocellulose membrane, and Hyperfilm ECL were purchased from Amersham (Oakville, Canada).

Immunoblotting of colonic homogenate. A suspension of colonic tissue was obtained as described above. Samples were taken from animals treated 4 h to 14 days previously with TNBS. Samples were resuspended in ice-cold homogenization buffer that consisted of 50 mM Tris·HCl (pH 7.5), 0.25 M sucrose, 2 mM EDTA, 1 mM EGTA, 25 μg/ml leupeptin, 25 μg/ml aprotinin, 1 μg/ml soybean trypsin inhibitor, 50 μg/ml PMSF, and 10 mM β-mercaptoethanol with 10% Triton. Homogenized samples were centrifuged at 25,000 *g* for 30 min. The supernatant was mixed with an equal volume of 2% SDS sample buffer (125 mM Tris, pH 6.8, 20% glycerol, and 10% mercaptoethanol) and heated at 95°C for 5 min. The protein concentration of each extract was subsequently determined. Each sample of 12–15 μg homogenized protein was subjected to 10% SDS-PAGE. After electrophoresis, the gels were soaked for 30 min in transfer buffer and electroblotted onto nitrocellulose membranes using Mini Trans-Blot (Bio-Rad) for 75 min at 100 V. After transfer, the membrane was incubated for 1 h with 10% nonfat dry milk in PBS and blots were incubated with either specific PKCα antibody (1:1,500) for 2 h or PKCβ$_{II}$, PKCδ, or PKCε antibodies (1:1,000) for 3 h at room temperature. HRP-linked secondary antibody (1:6,000) incubation was for 1 h at room temperature. The blots were washed three times (10 min each time) between each antibody step with 0.1% Tween-20 in PBS. The ECL kit was used to visualize the immunoreactive bands according to the manufacturer's protocols. The density of the immunoreactive bands on the autoradiogram was measured by Image Master VDS (Pharmacia Biotech). Band intensity was quantified by measurement of the absolute integrated optical density, which estimates the volume of the band in the lane profile as calculated by Image Master VDS software.

Statistical Analysis

Results are presented as means ± SE for *n* animals. Data were analyzed by ANOVA and Dunnett's or Newman-Keuls tests for multiple comparisons or by Student's *t*-tests for unpaired data; $P < 0.05$ was the minimum accepted level of significance for all groups.

RESULTS

Effect of TNBS or Ethanol on Mucosal Damage, PKC Activity, and MPO Activity

Intracolonic administration of TNBS (75 mg/kg) caused a time-related and significant ($P < 0.05$) increase in PKC activity between 4 h and 1 wk after administration (Fig. 1*A*). Elevated PKC activity sub-

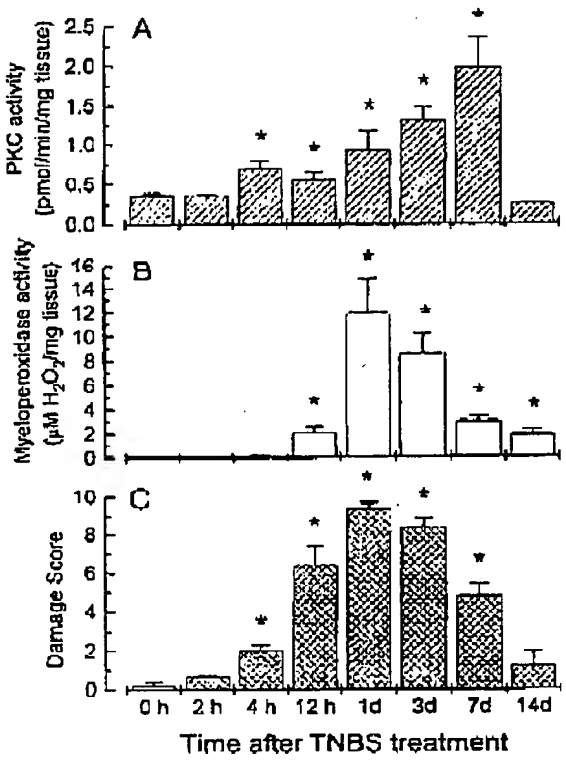


Fig. 1. Effect of 2,4,6-trinitrobenzenesulfonic acid (TNBS) treatment on means ± SE of mucosal protein kinase C (PKC) activity (*A*), mucosal myeloperoxidase (MPO) activity (*B*), and macroscopic damage score (*C*). Rats were treated with 75 mg/kg TNBS intracolonically (0.6 ml) and assessed at various times (2 h to 14 days) after treatment. *Significant difference vs. 0 h as determined by ANOVA and Newman-Keuls test for multiple comparisons (n = 6–8 animals/time point; $P < 0.05$).

sided between 7 and 14 days after administration of TNBS. Administration of TNBS also caused a time-related increase in MPO activity commencing 12 h after instillation. Activity, which was maximal at 1 day after TNBS administration, declined over the next 14 days (Fig. 1*B*). Similarly, macroscopic mucosal damage was evident as early as 4 h after TNBS instillation, with the degree of injury increasing to its maximum 1 day after administration of the hapten TNBS (Fig. 1*C*). Mucosal damage declined from that time so that by *day 14* the extent of damage was not significantly different from that observed in response to vehicle treatment alone.

In contrast, intracolonic treatment with the ethanol vehicle did not affect the activity of PKC in colonic mucosal homogenates (Fig. 2*A*). Both MPO activity and macroscopic mucosal damage were significantly elevated at 1 day (Fig. 2, *B* and *C*). Neither parameter was significantly affected at any other time point.

Effect of Staurosporine, GF-109203X, and Neutropenia on TNBS-Induced PKC Activity and Mucosal Damage

Administration of either staurosporine or GF-109203X (25 ng/kg iv; 30 min before induction of colitis by TNBS) each inhibited the increase in PKC activity observed 1 day after TNBS treatment (Fig. 3*A*). Furthermore, administration of the nonselectiv PKC antagonist staurosporine and the selective antagonist GF-109203X 30 min before induction of colitis with TNBS significantly reduced the macroscopic damage score assessed 24 h later (Fig. 3*B*). The extent of damage after staurosporine or GF-109203X treatment was significantly greater than that observed in the vehicle control group. Treatment of rats with ANS did not significantly affect either PKC activity or the extent of mucosal injury as determined 1 day after TNBS treatment.

Effect of PKC Inhibitor on Mucosal MPO Levels

Intravenous administration of either staurosporine or GF-109203X 30 min before TNBS treatment both significantly inhibited the increase in MPO levels observed in response to TNBS (1 day) alone, although these levels were still significantly greater than the MPO activity detected in vehicle control samples (Fig. 4).

Effect of PMA on Mucosal Damage

Intracolonic instillation of the PKC activator PMA in the concentrations of 1.5 and 3.0 mg/kg resulted in a

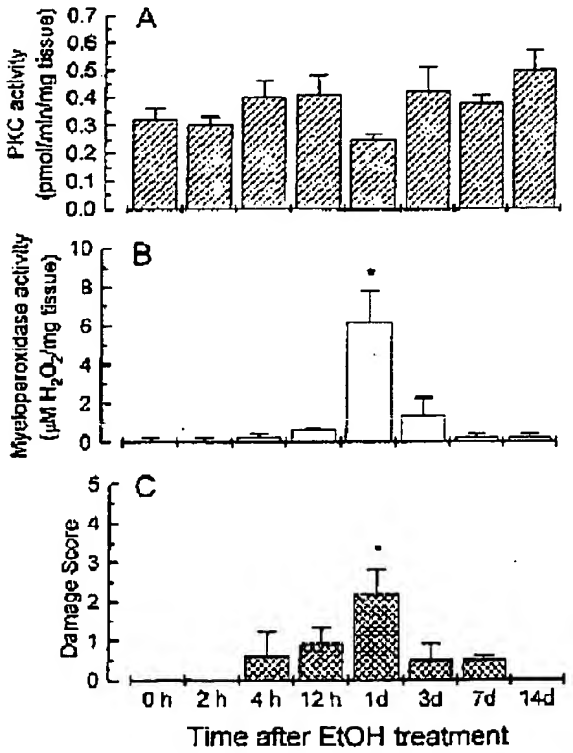


Fig. 2. Effect of ethanol (EtOH) treatment (50% wt/vol; 0.6 ml intracolonically) on means ± SE of mucosal PKC activity (*A*), MPO activity (*B*), and macroscopic damage score (*C*). Rats were assessed at various times (2 h to 14 days) after ethanol treatment. *Significant difference vs. 0 h as determined by ANOVA and Newman-Keuls test for multiple comparisons (n = 7–8 animals/time point; $P < 0.05$).

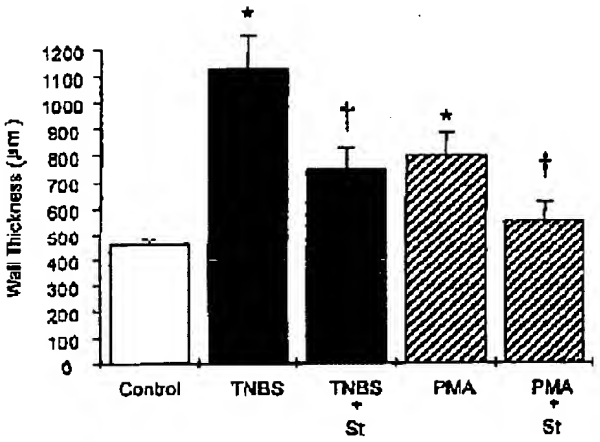


Fig. 6. Effect of TNBS (75 mg/kg) or PMA (3.0 mg/kg) treatment (intracolonically), with or without staurosporine (25 ng/kg iv) pretreatment on colonic wall thickness (means ± SE). Tissues were examined 1 day after TNBS or PMA instillation. *Significant increase over respective controls, †significant reduction from TNBS or PMA alone as determined by ANOVA and Newman-Keuls test for multiple comparisons (n = 6–8 animals/group; $P < 0.05$).

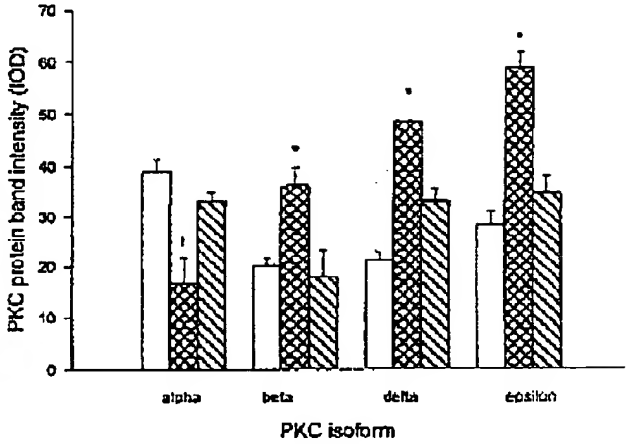


Fig. 8. Densitometric analysis of PKC protein content (means ± SE) in rat colonic tissue taken 1 day after intracolonic TNBS (cross-hatched bars) or ethanol (hatched bars) instillation. Western blot analysis for PKCα, PKCβ, PKCδ, and PKCε and densitometry were performed as described in MATERIALS AND METHODS. IOD, integrated optical density. *Significant increase, †significant decrease from respective controls (open bars) as determined by Student's t-test for unpaired data (n = 6–8 animals/group; $P < 0.05$).

DISCUSSION

In this study of experimentally induced colitis, we have attempted to determine whether there is a direct association between the severity of inflammation as estimated by myeloperoxidase activity, gross mucosal injury, and activation of PKC in the colonic mucosa. The study revealed that intrarectal instillation of TNBS resulted in a progressive increase in the severity of colitis commencing 2–4 h after instillation of TNBS, becoming maximal 1 day after induction, and then declining over the next 14 days. This pattern of colonic injury in response to TNBS has been described previously (2, 22).

This study also demonstrated that, in response to TNBS, mucosal PKC activity was elevated between 2 and 4 h and increased over the next 7 days. Furthermore, protein content for PKCε was also increased as early as 4 h after TNBS treatment. Treatment of rats with the ethanol vehicle alone did not affect PKC activity or the protein levels of PKCα, PKCβ, PKCδ, or PKCε at any time point. Furthermore, significant mucosal damage was only evident on *day 1*, and this level was significantly less than that observed on *day 1* after TNBS treatment. Therefore, ethanol-mediated mucosal injury was relatively short-lived and not dependent on PKC activation.

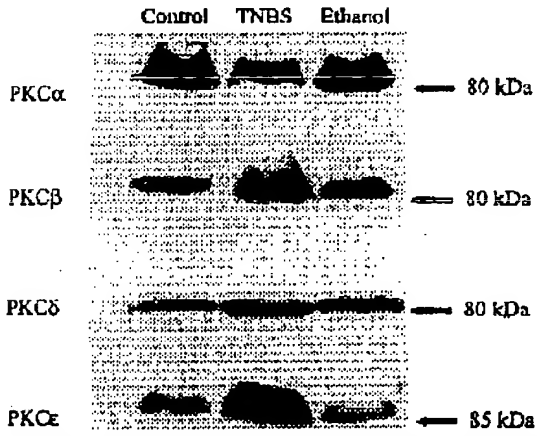


Fig. 7. Western blot of PKC isoforms in rat colonic mucosal scrapings taken 1 day after TNBS or ethanol treatment; 12–15 μg of protein were subjected to 10% SDS gel electrophoresis. Specific PKC bands can be seen at 80 kDa for PKCα, PKCβ, and PKCδ and at 85 kDa for PKCε.

The extent of mucosal injury in TNBS-treated animals was inhibited when animals were pretreated with the nonselective PKC antagonist staurosporine. Staurosporine treatment also reduced the increase in mucosal thickness in colonic tissues in which PKC activity had been augmented. Staurosporine has previously been shown to reduce injury induced in response to a number of inflammatory challenges in nongastrointestinal tissues and to inhibit release of certain proinflammatory mediators (12, 13, 21). In contrast to staurosporine, which inhibits not only PKC but also protein kinase A (PKA), protein kinase G, and myosin light chain kinase activity, GF-109203X is a potent and selective inhibitor of PKC activity with no effects on PKA or tyrosine kinase activities (39). In the present study, GF-109203X also reduced the extent of damage and inhibited PKC activity in tissue excised from TNBS-challenged animals. These data suggest that PKC plays a direct role in this type of experimentally induced colitis.

This suggestion is reinforced by our demonstration that intraluminal instillation of the PKC activator PMA could also produce mucosal damage, which was also inhibited by the antagonist staurosporine. PMA is a potent activator f inflammatory cells (36) that has

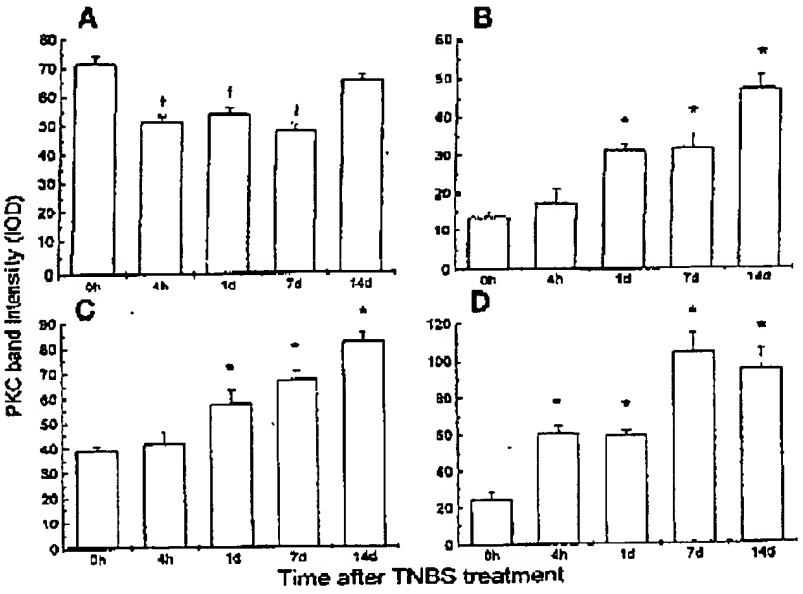


Fig. 9. Densitometric analysis of PKCα (*A*), PKCβ (*B*), PKCδ (*C*), and PKCε (*D*) protein content (means ± SE) in colonic mucosa from rats at various times (0–14 days) after TNBS treatment. *Significant increase, †significant decrease from 0 h as determined by ANOVA and Newman-Keuls test for multiple comparisons (n = 6–8 animals/group; $P < 0.05$).

been used to model various intestinal diseases. Intraluminal PMA instillation has been shown to produce colonic mucosal inflammation (3, 5, 9) and ileal microvascular injury (28), and recently PMA treatment has been associated with gastric ulcer formation (37). In the present study, PMA acted via a PKC-dependent mechanism as assessed using the PKC-inactive phorbol analog 4α-PMA. This confirms and extends findings by Berin and Buell (3) in which PMA but not the bioinactive analog increased colonic epithelial permeability.

Changes in PKC activity have been associated with inflammation of a number of tissues and cells, including skin, cartilage, and epithelial cells of the airway, glomerulus, and liver (10, 31, 35, 36). A number of isoforms of PKC are expressed in the colon (6), and PKC activity has been shown to be increased in tissue from ulcerative colitis patients (33). Furthermore, increased PKC activity is also linked with the increase in mesenteric venular leakage in response to ischemia-reperfusion injury to the intestine (43). PKC activation has also been shown to be associated with an increase in intestinal endothelial and epithelial permeability in vivo and in vitro (3, 27, 29). Those data together with the present results strongly suggest a causative role for PKC in the initiation of colonic mucosal injury.

The mechanisms through which increases in PKC activity mediate TNBS-induced colitis are unknown. PKC has been shown to regulate a variety of signal transduction events involved in inflammation, including induction of NO synthase activity, production of cytokines, oxidants, and activation of phospholipase A_2 (14, 34). PKC activation via PMA has also been shown to cause activation of neutrophils (18), resulting in an oxidative burst (8, 18). Although neutrophil infiltration has been associated with TNBS-induced colitis (2), neutrophil depletion has not been shown to effectively reduce acetic acid- or TNBS-induced colonic damage (5, 42). In our study, although TNBS treatment resulted in an increase in MPO activity, induction of neutropenia via antineutrophil serum administration did not ameliorate the TNBS-mediated colonic injury. Furthermore, neutropenia did not significantly reduce mucosal PKC activity. Similarly, in PMA-induced colitis, neutropenia has been shown to be ineffective in reducing the degree of damage (5). In contrast, the PKC antagonists staurosporine and GF-109203X reduced the increase in MPO levels in colonic mucosal samples from TNBS-treated rats. These data suggest that, in TNBS-induced colitis, neutrophil infiltration is secondary to tissue damage and the response is not mediated by PKC. However, the ability of the PKC antagonists to reduce tissue injury may be attributed in part to a reduction in neutrophil infiltration as well as a reduction in tissue PKC activity.

Normal colonic mucosa expresses a number of PKC isoforms with distinct subcellular distributions for each (6). In the present study using tissue from TNBS-challenged animals, we detected protein for PKCα, PKCβ, PKCδ, and PKCε. These PKC isoforms have been associated with cytotoxicity in some cell types (7), and these isoforms can be activated by proinflammatory cytokines (19). Furthermore, the results of work done in tissues such as liver, brain, and peripheral nerves and in cells such as macrophages indicate that an inflammatory challenge can result in changes in the expression of various PKC isoforms (16, 32, 34). The present results indicate that PKCβ, PKCδ, and PKCε are all elevated in response to TNBS treatment, although only PKCε protein is increased before the onset of colonic damage. PKCα content is decreased as early as 4 h after treatment and only returns to control levels at 14 days. It may be suggested that the elevated isoforms (PKCβ, PKCδ, and PKCε) play roles at various

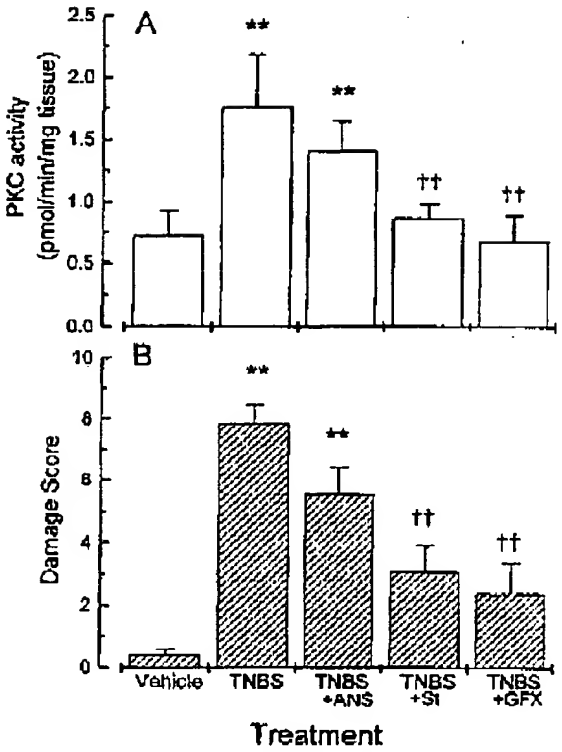


Fig. 3. Effect of TNBS treatment (75 mg/kg intracolonically) alone or with pretreatment with antineutrophil serum (ANS; 100 μl/kg ip), staurosporine (St; 25 ng/kg iv), or GF-109203X (GFX; 25 ng/kg iv) on means ± SE of mucosal PKC activity (*A*) and macroscopic damage score (*B*). Tissues were examined 1 day after TNBS treatment. **Significant difference vs. vehicle (50% ethanol) control, ††significant difference vs. TNBS treatment alone by ANOVA and Newman-Keuls test for multiple comparisons (n = 6–8 animals/group; $P < 0.05$).

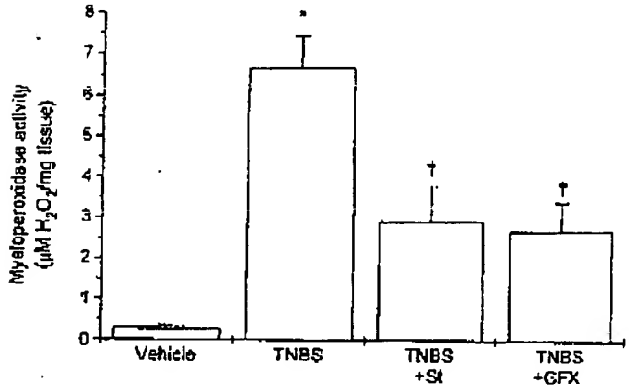


Fig. 4. Effect of TNBS treatment alone or with pretreatment with either staurosporine or GF-109203X (each at 25 ng/kg iv; 30 min before TNBS) on MPO activity (means ± SE). Tissues were examined 1 day after TNBS treatment. *Significant difference vs. vehicle control, †significant difference vs. TNBS alone by ANOVA and Newman-Keuls test for multiple comparisons (n = 6–7 animals/group; $P < 0.05$).

dose-dependent increase in mucosal injury. Preadministration of the PKC antagonist staurosporine significantly reduced PMA-induced colonic damage (Fig. 5). The bioinactive PMA analog 4α-PMA was without effect in these studies. As detailed in Fig. 6, colonic wall thickness was increased in rats examined 1 day after TNBS or PMA treatment. This was observed to be due primarily to edema, although in some animals muscularis thickness was enhanced as well. Staurosporine treatment significantly reduced wall thickness in TNBS-treated rats, although this parameter was still greater than in control animals. Staurosporine treatment significantly reduced wall thickness of PMA-treated rats to control levels (Fig. 6).

Effect of TNBS on PKC Isoforms

Immunoblot analysis of PKC revealed that TNBS treatment increased PKCβ, PKCδ, and PKCε protein. In contrast, PKCα protein was not augmented by TNBS treatment (Fig. 7). Densitometric analysis indicated that, although protein for the PKCβ, PKCδ, and PKCε isoforms increased 1 day after TNBS treatment, administration of the ethanol vehicle had no significant effect in this regard (Fig. 8). PKCα protein did not increase significantly over the 14 days of observation. Densitometric analysis of PKCα revealed that this isoform was significantly decreased from 4 h to 7 days after TNBS treatment and returned to control levels on *day 14* (Fig. 9*A*). PKCβ, PKCδ, and PKCε protein content all increased within 1 day after TNBS treatment, whereas PKCε was increased as early as 4 h after TNBS treatment (Fig. 9*D*). Protein levels for PKCβ, PKCδ, and PKCε remained elevated even at 14 days after TNBS treatment.

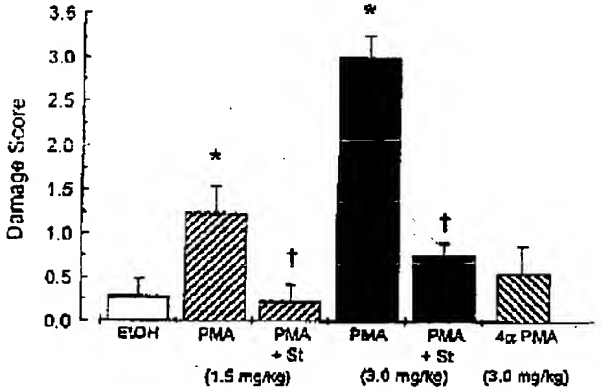


Fig. 5. Effect of intracolonic instillation (0.6 ml) of PKC activator phorbol 12-myristate 13-acetate (PMA; 1.5–3.0 mg/kg in 20% ethanol) on macroscopic mucosal damage score (means ± SE). In some experiments, animals were pretreated with PKC inhibitor staurosporine (25 ng/kg iv). In other studies, rats were treated with bioinactive PMA analog 4α-phorbol 12-myristate 13-acetate (4α-PMA; 3.0 mg/kg). Tissues were examined 1 day after PMA treatment. *Significant increase over respective controls, †significant reduction from PMA treatment alone as determined by ANOVA and Dunnett's test (n = 6–8 animals/group; $P < 0.05$).

stages of TNBS-induced inflammation. The return of PKCα to control levels at *day 14* suggests that this isoform contribut s to the reduced PKC activity measured at that time point and may play a role in the restitution process.

In summary, our results provide the first evidence that PKC is directly involved in colonic mucosal inflammation. This study also supports the proposal that neutrophils do not mediate the inflammatory effects of TNBS and are not involved in the colonic response to PKC activation.

This work was supported by Medical Research Council of Canada Grant MT 6426. J. F. Brown was supported by a Canadian Association of Gastroenterology/Astra Research Initiative Award.

Address for reprint requests: B. L. Tepperman, Dept. of Physiology, Faculty of Medicine, University of Western Ontario, London, ON, Canada N6A 5C1 (E-mail: btepper@physiology.uwo.ca).

Received 16 June 1998; accepted in final form 4 November 1998.

REFERENCES

1. **Akiguchi, I., M. Izumi, and S. Nagataki.** Effects of phorbol ester on protein kinase C activity are effects of depletion of its activity on thyrotropin, forskolin, and 8' bromoadenosine 3'5' cyclic-monophosphate-induced [^{3}H]thymidine incorporation in rat FRTL-5 cells. *J. Endocrinol.* 138: 379–389, 1993.
2. **Bell, C. J., D. G. Gall, and J. L. Wallace.** Disruption of colonic electrolyte transport in experimental colitis. *Am. J. Physiol.* 268 (*Gastrointest. Liver Physiol.* 31): G622–G630, 1995.
3. **Berin, M. C., and M. G. Buell.** Phorbol myristate acetate ex vivo model of enhanced colonic epithelial permeability. Reactive oxygen metabolitic and protease independence. *Dig. Dis. Sci.* 40: 2268–2279, 1995.
4. **Brawn, M. K., W. J. Chiou, and K. L. Leach.** Oxidant-induced activation of protein kinase C in UC11MG cells. *Free Radic. Res.* 22: 23–37, 1995.
5. **Buell, M. G., and M. C. Berin.** Neutrophil independence of the initiation of colonic injury. Comparison of results from three models of experimental colitis in the rat. *Dig. Dis. Sci.* 39: 2575–2588, 1994.
6. **Davidson, L. A., Y. H. Jiang, J. N. Derr, H. M. Aukema, J. R. Lupton, and R. S. Chapkin.** Protein kinase C isoforms in human and rat colon. *Arch. Biochem. Biophys.* 312: 547–553, 1994.
7. **De Vente, J., S. Kiley, T. Garris, W. Bryant, V. Hooker, K. Posekany, P. Parker, P. Look, D. Fletcher, and D. K. Ways.** Phorbol ester treatment of U937 cells with altered protein kinase C content and distribution induces cell death rather than differentiation. *Cell Growth Differ.* 6: 371–382, 1995.
8. **English, D., J. S. Roloff, and J. N. Lakens.** Chemotactic factor enhancement of superoxide release from fluoride and phorbol myristate acetate stimulated neutrophils. *Blood* 58: 129–134, 1981.
9. **Fretland, D. J., D. L. Widomski, S. Levin, and T. S. Gaginella.** Colonic inflammation in the rabbit induced by phorbol-12-myristate-13-acetate. *Inflammation* 14: 143–150, 1990.
10. **Geng, Y., R. Maier, and M. Lotz.** Tyrosine kinases are involved with the expression of inducible nitric oxide synthase in human articular chondrocytes. *J. Cell. Physiol.* 163: 545–554, 1995.
11. **Goldman, R., E. Ferber, and U. Zort.** Reactive oxygen species are involved in the activation of cellular phospholipase A_2. *FEBS Lett.* 30: 190–192, 1992.
12. **Hara, H., H. Onodera, M. Yoshidomi, Y. Matsuda, and K. Kogure.** Staurosporine, a novel protein kinase C inhibitor, prevents postischemic neuronal damage in the gerbil and rat. *J. Cereb. Blood Flow Metab.* 10: 646–653, 1990.
13. **Hirasawa, N., M. Shiraishi, N. Tokuhara, Y. Hirano, A. Mizuntari, S. Mue, and K. Ohuchi.** Pharmacological analysis of the inflammatory exudate-induced histamine production in bone marrow cells. *Immunopharmacology* 36: 87–94, 1997.
14. **Jacobson, P. B., S. L. Kuchera, A. Metz, C. Schachtele, K. Imre, and D. J. Schrier.** Antiinflammatory properties of GO 6850: a selective inhibitor of protein kinase C. *J. Pharmacol. Exp. Ther.* 275: 995–1002, 1995.
15. **Jan, C.-D., B.-M. Choi, H. Ryn, J.-Y. Um, H.-J. Kwak, B.-S. Lee, S.-G. Pak, H.-M. Kim, and H.-T. Chung.** Synergistic co-operation between phorbol ester and IFN for induction of nitric oxide synthesis in murine peritoneal macrophages. *J. Immunol.* 153: 3684–3690, 1994.
16. **Jones, B. A., Y. P. Rao, R. T. Stravitz, and G. J. Gores.** Bile salt-induced apoptosis of hepatocytes involves activation of protein kinase C. *Am. J. Physiol.* 272 (*Gastrointest. Liver Physiol.* 35): G1109–G1115, 1997.
17. **Kuruvilla, A., G. Putcha, E. Poulos, and W. T. Shearer.** Tyrosine phosphorylation of phospholipase C concomitant with its activation by platelet-activating factor in a human B cell line. *J. Immunol.* 151: 637–648, 1993.
18. **Lehrer, R. I., and L. Cohen.** Receptor-mediated regulation of superoxide production in human neutrophils stimulated by phorbol myristate acetate. *J. Clin. Invest.* 68: 1314–1320, 1981.
19. **Linatola, C., B. Barabino, A. Nistn, and A. Santoni.** Interleukin 1β induced protein kinase C-ξ activation is mimicked by exogenous phospholipase D. *Biochem. J.* 320: 497–501, 1987.
20. **Louise, C. B., M. C. Tran, and T. G. Obrig.** Sensitization of human umbilical vein endothelial cells to shiga toxin: Involvement of protein kinase C and NFκB. *Infect. Immun.* 65: 3337–3344, 1997.
21. **Miesel, R., D. Sanocka, M. Kurpisz, and H. Kroger.** Antiinflammatory effects of NADPH oxidase inhibitors. *Inflammation* 19: 347–362, 1995.
22. **Morris, G. P., P. L. Beck, M. S. Herridge, W. T. Depew, M. R. Szewdzuk, and J. L. Wallace.** Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 96: 795–803, 1989.
23. **Newton, A. C.** Protein kinase C: structure, function and regulation. *J. Biol. Chem.* 270: 28495–28498, 1995.
24. **Nishizuka, Y.** Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C. *Science* 258: 607–614, 1992.
25. **Nishizuka, Y.** Protein kinase C and lipid signalling for sustained cellular responses. *FASEB J.* 9: 484–496, 1995.
26. **Node, K., M. Kitakaze, T. Minamino, M. Tada, M. Inoue, M. Hori, and T. Kamada.** Activation of ecto-5'-nucleotidase by protein kinase C and its role in ischaemic tolerance of the canine heart. *Br. J. Pharmacol.* 120: 273–281, 1997.
27. **Northover, A. M., and B. J. Northover.** Stimulation of protein kinase C activity may increase microvascular permeability to colloidal carbon via alpha-bioenzyme. *Inflammation* 18: 481–487, 1994.
28. **Overdahl, M. C., M. W. Julian, S. E. Welsbrode, and P. M. Dorinsky.** Anti-CD18 antibody does not block ileal injury induced by phorbol myristate acetate. *Am. J. Respir. Crit. Care Med.* 152: 1331–1336, 1995.
29. **Perez, M., A. Barber, and F. Ponz.** Modulation of intestinal paracellular permeability to intracellular mediators and cytoskeleton. *Can. J. Physiol. Pharmacol.* 75: 287–292, 1997.
30. **Phelps, D. T., T. J. Ferro, R. J. Higgins, R. Shankar, D. M. Parker, and A. Johnson.** TNFα induces peroxynitrite-mediated depletion of lung endothelial glutathione via protein kinase C. *Am. J. Physiol.* 269 (*Lung Cell. Mol. Physiol.* 13): L551–L559, 1995.
31. **Post, G., and J. H. Brown.** G protein-coupled receptors and signaling pathways regulating growth responses. *FASEB J.* 10: 741–749, 1996.
32. **Roberts, R. E., and W. G. McLean.** Protein kinase C isozyme expression in sciatic nerves and spinal cords of experimentally diabetic rats. *Brain Res.* 754: 147–156, 1997.
33. **Sakamoue, Y., T. Hatada, T. Horai, Y. Shoji, M. Kusumoki, and J. Utsunomiya.** Protein kinase C activity of colonic mucosa in ulcerative colitis. *Scand. J. Gastroenterol.* 27: 275–280, 1992.
34. **Shapiro, L., V. L. Sylvia, A. Halabi, W. A. Soskolne, T. E. Van Dyke, D. D. Doon, B. D. Boyan, and Z. Schwartz.** Bacterial lipopolysaccharide induces early and late activation of protein kinase C in inflammatory macrophages by selective activation of PKC-epsilon. *Biochem. Biophys. Res. Commun.* 240: 629–634, 1997.

35. **Sharma, K., T. M. Danoff, A. De Piero, and F. N. Ziyadeh.** Enhanced expression of inducible nitric oxide synthase in murine macrophages and glomerular mesangial cells by elevated glucose levels: possible mediation via protein kinase C. *Biochem. Biophys. Res. Commun.* 207: 80–88, 1995.
36. **St. John, R. C., L. A. Mizer, S. E. Weisbrode, and P. M. Dorinsky.** Increased intestinal protein permeability in a model of lung injury induced by phorbol myristate acetate. *Am. Rev. Respir. Dis.* 144: 1171–1176, 1991.
37. **Takeuchi, T., S. Miura, R. Hokari, I. Kurose, Y. Akiba, M. Hirokawa, H. Higuchi, S. Zeki, M. Adachi, H. Kimura, and H. Ishi.** Gastric ulcer produced by phorbol myristate acetate: role of leukocyte adhesion and activation of nuclear factor kappa B (Abstract). *Gastroenterology* 112: A308, 1997.
38. **Tepperman, B. L., B. L. Vozzolo, and B. D. Soper.** Effect of neutropenia in gastric mucosal integrity and nitric oxide synthesis in the rat. *Dig. Dis. Sci.* 38: 2056–2061, 1993.
39. **Toullec, D., P. Pianetti, H. Coste, P. Bellevergue, T. Grand-Perret, M. Ajakane, V. Baudet, P. Boisson, E. Boursier, F. Loriolle, L. Duhamel, D. Charon, and J. Kirilovsky.** The bisindoyl maleimide GF 109203X is a potent and selective inhibitor of protein kinase C. *J. Biol. Chem.* 266: 15771–15781, 1991.
40. **Wallace, J. L.** Glucocorticoid-induced gastric mucosal damage: inhibition of leukotriene but not prostaglandin biosynthesis. *Prostaglandins* 34: 311–323, 1987.
41. **Ward, C. A., and M. P. Moffat.** Role of protein kinase C in mediating effects of hydrogen peroxide in guinea pig ventricular myocytes. *J. Mol. Cell. Cardiol.* 27: 1089–1097, 1995.
42. **Yamada, T., B. J. Zimmerman, R. D. Specian, and M. B. Grisham.** Role of neutrophils in acetic acid-induced colitis in rats. *Inflammation* 15: 399–411, 1991.
43. **Yarimizu, K., T. Ishida, C. Gute, and R. J. Korthius.** Role of protein kinase C in ischemia/reperfusion-induced venular protein leakage in the rat mesentery (Abstract). *Gastroenterology* 112: A422, 1997.
44. **Ye, X., I. Georgoff, S. Fleisher, F. D. Coffman, S. Cohen, and K. L. Fresa.** The mechanism of epipodophyllotoxin-induced thymocyte apoptosis: possible role of a novel Ca^{2+}-independent protein kinase. *Cell. Immunol.* 151: 320–335, 1993.

EXHIBIT "E"

<1>

Unique Identifier
96133430

Authors
Melarange R. Gentry C. Toseland CD. Smith PH. Fuller J.

Title
Neutropenia does not prevent etodolac- or indomethacin-induced gastrointestinal damage in the rat.

Source
Digestive Diseases & Sciences. 40(12):2694-703, 1995 Dec.

Local Messages
Hardin Library has current subscription

Abstract
Neutrophils have been implicated in the acute formation of gastric mucosal erosions induced by nonsteroidal antiinflammatory drugs. The aims of the present study were to determine, in rats, the role of neutrophils in the pathogenesis of etodolac- and indomethacin-induced gastrointestinal ulceration and blood loss. Both drugs caused gastrointestinal ulceration, which was associated with increased blood loss, a rise in plasma haptoglobin concentration, and a rise in the number of circulating neutrophils. A marked infiltration of neutrophils occurred only in ileal tissue. Pretreatment with a selective antineutrophil serum induced a significant neutropenia, which failed to inhibit either etodolac- or indomethacin-induced gastrointestinal ulceration and blood loss. A further study demonstrated that the antineutrophil serum did not prevent gastric erosions induced by indomethacin, but it inhibited carrageenan paw edema, which is dependent, in part, on neutrophil infiltration and activation. It is concluded that neutrophils do not contribute to gastrointestinal ulceration and blood loss induced by nonsteroidal antiinflammatory drugs. Furthermore, in contrast with previous studies, our results provide no evidence that neutrophils contribute to indomethacin-induced acute gastric erosion formation.